TRANSLOCATION OF NEONICOTINOID IN MAIZE AND NONTARGET IMPACTS TO WATER AND SOIL

by

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ABSTRACT

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The neonicotinoids are a relatively new group of insecticides, first commercially available in the early 1990s, and have become the most widely used insecticide class in the world. Neonicotinoids can be utilized in a variety of ways however the vast majority are applied as a seed coating prior to planting. These neonicotinoid seed treatments (NST) are primarily used within field crops, where their main use is as a prophylactic, insurance based approach to pest management. By 2011 US adoption rates exceeded >80% in maize and 34-44% in soy. This widespread and rapid use has not been justified with a corresponding rise in pest pressure. Numerous peer-reviewed publications had reported inconsistent yield benefits for NST use at the start of this dissertation. Furthermore, an increasing number of reports have reported contamination of nontarget areas with neonicotinoids.

In order to investigate one potential mechanism to explain both inconsistent yield benefits and estimate the contribution of NST to environmental contamination, I conducted a two-year field study in which I compared concentrations of clothianidin seed treatments in maize to that of maize without neonicotinoid seed treatments. I found the protection of NST extends to at least 34 d post planting and that in-plant concentrations followed an exponential decay pattern with initially high values followed by a rapid decrease within the first ~20 d post planting. A cumulative maximum of 1.34% of the initial seed treatment was successfully recovered from whole plant tissues in both study years with only 0.26% of the initial seed treatment being recovered from root tissue. My findings suggest NST may provide some protection from early season maize pests but, even at the highest per kernel concentrations of clothianidin tested, peak in-plant concentrations are poorly correlated with the key pest that they are labeled to control: the corn rootworm the key maize pest within the US. Additionally, the poor translocation efficiency of NST, in combination with the high leaching risk of these compounds, provides a route for environmental contamination.

To further address the crop protection efficacy of NST, and determine if their mandatory use is justified (growers usually do not have access to untreated seed), I also conducted a meta-analysis on plant health and pest damage metrics from 15 yr of insecticide efficacy trials conducted on Indiana maize. Corn rootworm remains the key pest of maize in the United States however it is managed largely by Bt corn hybrids, along with soil insecticides and NSTs. Frequently, more than one of these pest-management approaches is employed at the same time. This meta-analytical approach allowed me to summarize the mean effect over 15 yrs worth of data and pest management techniques. The probability of recovering the insecticide cost associated with each treatment was also calculated when possible. With the exception of early-season plant health (stand counts), in which the NSTs performed better than all other insecticides, the vast majority of insecticides performed similarly in all plant health metrics, including yield. Furthermore, all tested insecticides (including NSTs) reported a high probability (>80%) of recovering treatment costs. Given the similarity in performance and probability of recovering treatment costs, I suggest NSTs be optional for producers, so that they can be incorporated into an insecticide rotation when managing for corn rootworm, the primary Indiana corn pest. This approach could simultaneously reduce costs to growers, lower the likelihood of non-target effects, and reduce the risk of pests evolving resistance to the neonicotinoid insecticides.

The high soil half-life and low K_{ow} of many neonicotinoids result in a high leaching risk. This, in combination with their annual repeated use is likely the reason an increasing number of detections of environmental neonicotinoids are being reported. In order to quantify the magnitude and timing of CLO concentrations in leachate from tile drain fields, an experiment was carried out using field lysimeters at the Purdue Water Quality Field Station. The maximum clothianidin concentration in leachate was found to be an approximate order of magnitude higher than previously reported (3.48 ng/ml). This is not surprising, as these data result from a direct and undiluted measure of neonicotinoid leachate reinforcing the role of NST to environmental contamination. Furthermore, my work demonstrates that NST concentrations within leachate are greatest at precipitation events that follow planting and conform to a first-order decay pattern of initially high concentrations, with a rapid and drastic concentration decrease as the growing season progresses. I also investigated the possibility of nontarget impacts resultant from neonicotinoid contamination in leachate. The systemic nature of NST allows them to readily be translocated by nontarget vegetation and at the start of this dissertation, non-target translocation had not been

reported in aquatic vegetation. This question was explored with manipulative laboratory experiments to assess the uptake potential of aqueous clothianidin, a proxy for agricultural runoff and leachate, in the aquatic macrophyte, gibbous duckweed (*Lemna gibba*). Clothianidin was found to reach equilibrium within plant tissues by 12 hrs exposure at a concentration ~65% of the concentration within the water. Finally, bioassays utilizing clothianidin-contaminated duckweed were conducted on a duckweed-associated insect (*Rhopalosiphum nymphaeae*, waterlily aphid (Linnaeus)) to investigate potential impacts on higher trophic levels

CHAPTER 1: INTRODUCTION

1.0 The neonicotinoids and their uses

The neonicotinoids are a relatively new group of insecticides, first commercially available in the early 1990s, and have become the most widely used insecticide class in the world (Goulson 2013). This group gets its name from the nicotine compounds within the plant family Solanaceae that their chemical structures are based upon. The neonicotinoids are most effective when delivered orally to a pest via ingestion of treated plant tissue and are less effective as contact insecticides. Neonicotinoids act as nicotinic acetylcholine receptor agonists by binding to and overstimulating nicotinic acetylcholine receptors (nAChRs) leading to paralysis and ultimately death. Because nAChR binding is much stronger in insects in comparison with mammals, the neonicotinoids exhibit reduced mammalian toxicity (Tomizawa and Casida 2005). The successful adoption of the neonicotinoids has been attributed to a combination of low mammalian toxicity, systemic and translaminar properties, lack of insect resistance upon market entry, increasing restrictions and regulations on older pesticide groups, and a wide range of possible application methods (Elbert et al. 2008).

Neonicotinoids can be utilized in a variety of ways, which includes foliar sprays, trunk injections, as an additive to irrigation water, and even as a topical treatment for household pets (Schenker et al. 2003, Elbert et al. 2008, Goulson 2013). Despite this, the vast majority of neonicotinoids are applied as a seed coating prior to planting within the developed world (Goulson 2013) where in 2005, they comprised 77% of the seed treatment market (Elbert et al. 2008). These neonicotinoid seed treatments (NST) are mainly used within the field crops, where their main use is as a prophylactic, insurance based approach to pest management with US adoption rates exceeding >80% in maize and 34-44% in soy by 2011 (Douglas and Tooker 2015, Krupke et al. 2012). Both thiamethoxam (TMX), and its breakdown product clothianidin (CLO), are solely used as STs in US maize, whereas foliar and ST options of CLO, TMX, and imidacloprid exist for soy systems. CLO or TMX are applied at rates of 0.25-1.25 mg of compound per maize kernel prior to being sold to the grower. However as of 2013, the 0.5 and 1.25 mg of active ingredient (AI) per maize kernel became more widely used, with one seed company only selling seed at the 1.25 mg AI ingredient kernel^{-1} rate (Douglas and Tooker 2015).

1.1 Integrated Pest Management in maize and the role of neonicotinoid seed treatments

The definition of integrated pest management (IPM) has evolved over the 50+ years since its inception (Kogan 1998). An ideal modern IPM program should include monitoring and management of key pests (including weeds, insects, and fungal pathogens), followed by the use of multiple suppressive tactics in order to achieve economic levels of control, and finally the judicious use of pesticides when/where necessary as determined by economic thresholds (Ehler 2006). In reality, modern IPM in field crops such as maize and soybeans frequently falls short of this ideal, relying largely on prophylactic pesticide use to treat pests and limited rotation of active ingredients to mitigate resistance (Ehler 2006).

NST have been proposed as a more targeted, and IPM friendly approach to crop protection as their use takes advantage of the systemic property of the insecticide. When applied to the soil or seed, neonicotinoids are translocated through plant roots where they are then distributed via xylem movement throughout the plant, providing systemic protection for a variable period of time, depending on the plant species, pesticide concentration, and environmental factors (Elbert et al. 2008). Using systemic insecticides as a component of pest management is not a novel concept with the first systemic compounds appearing in the 1950s. These included the soluble organophosphates (OP) dimethoate, demeton-S-methyl, mevinphos and phorate. Additional systemic pesticides were developed in the 1960s with the creation of the systemic carbamates aldicarb and carbofuran (Sánchez-Bayo et al. 2013). Since the passing of the Food Quality Protection Act (FQPA) enacted in 1996 (US-EPA 2002), many of the OPs and carbamates have experienced decreased use. For example of the 49 OPs registered for agricultural and residential use in 1996, 14 were removed from the market and 28 underwent increased risk mitigation efforts by 2002. Removal of these commonly used insecticides, along with the FQPA's focus on reduced-risk pesticides have allowed the neonicotinoids to fulfill an important niche in crop production. Despite this, the neonicotinoids are fulfilling novel roles, particularly as seed treatments and are deployed on a much wider scale than carbamates and OPs ever were.

1.2 US Maize production and pests

The US is the number one producer of maize worldwide with approximately 36.5 million hectares allocated for maize production alone in 2017 (USDA-NASS 2018). As previously mentioned, US adoption rates of NST exceeded >80% in maize by 2011 and have likely approached 100% (Douglas and Tooker 2015). This rapid and widespread adoption is likely due to a combination of increasing restrictions and regulations on older pesticide groups (Elbert et al. 2008), as well as marketing choices by seed companies as no increase in pest pressure has been reported (Krupke et al. 2012, Douglas and Tooker 2015). The NST are labeled to control the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (CRW), the primary insect pest in North American maize production, as well as other, secondary, early season pests including the wireworms (Riley and Keaster 1979), seedcorn maggots (Higley and Pedigo 1984), and white grubs (Jordan et al. 2012). Seedcorn maggots and wireworms preferentially attack the seed region of young plants early in the season (Riley and Keaster 1979, Higley and Pedigo 1984), whereas white grubs and CRW attack the roots (Metcalf and Metcalf 1993) causing plant lodging, reducing water uptake and ultimately increasing potential for yield loss (Levine and Oloumi-Sadeghi 1991). However, the vast majority of these secondary pests are not relevant to most producers within the cornbelt as economic infestations are erratic and difficult to predict (Royer et al. 2004). Seedcorn maggot may be a notable exception, with economic populations that can be somewhat reliably anticipated following the incorporation of a green cover crop into soil (Hammond 1990).

1.3 Efficacy of NST in suppressing maize pests

At the outset of this dissertation, peer-reviewed literature increasingly reported inconsistent yield benefits associated with NST use in maize (Cox et al. 2007, Wilde et al. 2007, Jordan et al. 2012, Petzold-Maxwell et al. 2013). Cox et al. (2007) found no significant yield differences, even at a significance threshold of $P = 0.10$, between an untreated control and two rates of CLO seed treatment (control, 0.25 mg kernel^{-1}, 1.25 mg kernel^{-1}) over a three-year period in New York. The authors concluded that CLO seed treatments were unnecessary when maize follows soybean in the region. Another three-year study investigated yield benefits of NST (CLO and TMX) at numerous locations in Kansas under very low pest pressure (Wilde et al. 2007). Significant differences $P < 0.05$) in yield were only observed at 7 of 19 sites in 2004, 3 of 8 sites in 2005, and 4 of 8 sites in 2006 with control plots occasionally producing higher yields than treated plots. A three-year study in Virginia (Jordan et al. 2012) was conducted to test varying fall sampling methods in an attempt to predict spring white grub infestations. Two different treatment levels of CLO and a control (untreated, 0.25 mg kernel^{-1}, and 1.25 mg kernel^{-1}) were used. At the conclusion of the study, the

1.25 mg kernel^{-1} treatment was found to increase maize stand counts in two of the three years in fields with above-threshold grub densities. However, an increase in yield was only reported in one of the three years in fields with above-threshold densities. No yield differences were reported at below-threshold fields. Finally, another two-year study (Petzold-Maxwell et al. 2013) investigated the effect of CLO seed treatments, granular soil insecticides (tebupirimphos and cyfluthrin) alone and in combination with Bt maize (expressing the insecticidal toxin Cry3Bb1) in controlling CRW. Study plots were placed in fields where a trap crop was grown the previous year to increase rootworm pressure. Four of the five tested sites reported average root node injury scores in the non-Bt, insecticide-free plots to be above 0.5 on the 0-3 node injury scale (Oleson et al. 2005). Values over 0.5 on this scale may result in economic losses (Gray and Steffey 1998, O'Neal et al. 2001) and this value is frequently used as a nominal threshold. In non-Bt plots, all insecticides decreased root injury but no differences were present in yield between Bt + insecticide plots versus Bt only plots. The authors of this study concluded with a recommendation against combining Bt maize with insecticides (including NST) since no clear benefit exists for the combined approaches. Reviewing the combined outcomes of these four studies reveals NST provide slight (Wilde et al. 2007, Jordan et al. 2012) to zero (Cox et al. 2007) yield benefit, and are likely redundant when used alongside other control approaches (Petzold-Maxwell et al. 2013, Alford and Krupke 2018)

It was not until Tinsley et al. (2015), that a robust and large-scale data set was examined to compare the efficacy of NST to that of other management approaches, specifically the use of soil insecticides and/or Bt hybrids. These investigators utilized Illinois and Nebraska maize insecticide efficacy trial data from 2003-14 to describe the damage reduction attributable to various management strategies. In comparison to untreated controls, root damage was reduced by 48% with NST at 1.25 mg AI/kernel, 72% with soil insecticides regardless of application rate AI, 78% for single trait Bt maize, and 90% for dual trait Bt maize (Tinsley et al. 2015). Tinsley et al. (2015) concluded that, given the other options available, NST are not suitable for CRW control and suggested rotation of single trait Bt maize with soil insecticide as an optimal crop protection and resistance management strategy. Two additional studies utilizing large datasets soon followed: North et al. (2017) and Alford and Krupke (2018). North et al. 2017 analyzed 91 NST trials from four states (Arkansas, Louisiana, Mississippi, Tennessee) from 2001-2014. While the number of trials in which significantly higher yields as a result of NST use was not reported, significant increases in both yield and economic returns were reported in 8 of the 14 total years when trials were grouped and analyzed by year. When trials were grouped and analyzed by state, Louisiana and Mississippi reported a significant yield increase, and of those two, an economic benefit was only reported from Mississippi. The final paper addressing this question (Alford and Krupke 2018) resulted from a portion of this dissertation (Chapter 3) and used a meta-analytical approach to analyze data from insecticide efficacy trials conducted across Indiana from 2000-2015. Treatments were separated by both AI and application rate. Using this meta-analytical approach, NSTs were found to perform just as well as at-planting soil insecticides, in protecting yield and roots (31 and 40 site years used in each analysis respectively) from pest damage as determined from the mean effect sizes and high degree of overlap between effect size confidence intervals. Finally, yield data in combination with the average Indiana yield from 2010-2015 were used to calculate probability of economic return for relevant insecticides. All tested insecticides had a high probability (>80%) of economic return. As such, Alford and Krupke (2018) ultimately concluded NST could easily be rotated in with other insecticides as a CRW management strategy given the similarity in performance, and that deploying them in combination with other approaches was not likely to offer economic benefit.

While the analyses conducted by Tinsley et al (2015) and Alford and Krupke (2018) are both derived from robust datasets, their conclusions are completely different. An explanation was not provided for the poor performance of NSTs in Tinsley et al. (2015) but it is likely the result of the greater CRW pressure experienced by the states (Illinois and Nebraska) used in their analyses. Corn rootworms are specialists, feeding almost exclusively upon commercially grown maize hybrids in North America. Illinois and Nebraska have been, respectively, the second and third largest producers of maize for the last 20+ years whereas Indiana has alternated between the 5th and 6th largest producer (USDA-NASS 2018). Perhaps more importantly, a greater portion of both Illinois and Nebraska farmland leans towards continuous maize cropping whereas Indiana has a greater adoption of maize/soy rotation. An estimate of cropping patterns can be derived from satellite data accessible on the USDA-NASS CropScape website (Han et al. 2012) using ERDAS imagine software (ERDAS IMAGINE 2016). Of all the Nebraska and Illinois hectares that produced maize at least once in nine growing seasons (2008-2016), 5.1% and 4.3% , respectively produced maize in eight of those years, and 4.4% and 2.6% for nine years. Under the same conditions, 2.2% and 1.2% of hectares in Indiana grew maize for eight and nine years, respectively. In general, estimates derived from this pixel counting approach tend to be downward-biased when compared with official estimates conducted by the USDA-NASS (Gallego 2004) and consequentially are not suitable for area estimation. Still, the clear trend towards continual maize cropping in the western corn belt, coupled with the overall greater area set aside for maize production, may explain why NSTs performed poorly in Tinsley et al. (2015), due to the overall greater CRW pressure. As such, the rotation recommendations of Alford and Krupke (2018) can largely only be applied to areas in which crop rotation is routinely practiced.

1.4 Environmental fate of neonicotinoid seed treatments in maize

Maize sowing in the US is typically achieved with tractor-drawn planters that use forced air systems to pick up individual seeds and drop them into a planting furrow. As seed treatments can cause seeds to stick together, resulting in uneven planting, talc or graphite are often added to seed boxes as a lubricant. This lubrication talc exhaust contains a small fraction (<2%) of the total active ingredient due to abrasion of seeds during transport and handling (Tapparo et al. 2012). While planting, the exhaust air from the forced air planters disperses the contaminated talc as a dust either down towards the soil or into the air where it can travel long distances (> 100 m beyond field margins) before settling (Krupke et al. 2017).

Once within the soil, the goal of NST is to provide systemic protection as the plant grows, however in reality, protection is provided by only a fraction of the applied AI. Applications of imidacloprid seed treatments, a compound no longer used as a seed treatment in maize, resulted in only ~20% of the seed applied active ingredient being translocated into the target plant (Sur and Stork 2003). The abiotic impacts of UV photolysis and leaching on the breakdown of soil bound imidacloprid were likely reduced to eliminated as the study was conducted within a greenhouse setting. Notably, the authors mentioned that uptake was likely exaggerated due to the biomass of the maize roots in comparison to the amount of soil in the planter boxes. This registrant-funded study was the only estimate of translocation efficiency in maize prior to the start of this dissertation work. A portion of this dissertation (Chapter 2) has since then been published (Alford and Krupke 2017) and found translocation efficiency to be <1.5% of the total AI applied to the seed, in this case CLO, into the target maize plant. This also remains the only peer-reviewed research paper detailing NST translocation efficiency in any crop grown under field conditions. The remaining ~96% of the applied active ingredient presumably enters the water column and/or soil matrix (Goulson 2013).

Once neonicotinoids enter the soil matrix, they are relatively stable and can persist for multiple seasons following use. The time required to dissipate half the active ingredient (DT_{50}) under field conditions ranges from 277-1386 (US-EPA 2010) and 7.1-92.3 (Hilton et al. 2016) days for CLO and TMX, respectively. While degradation and water-facilitated movement are presumably the two major routes by which neonicotinoids are lost from soil, the relative importance of either factor is currently unknown (Goulson 2013). Given the highly limited access to untreated seed (Douglas and Tooker 2015, Alford and Krupke 2018) soils in soy and maize rotation are likely exposed to a continual and repeated dose of neonicotinoid. Both CLO and TMX are hydrolytically stable with high solubilities of 0.327 g L^{-1} at 20°C and 4.1 g L^{-1}, respectively, at 25°C. Furthermore, the Groundwater Ubiquity Score (GUS), while not the only metric used in leaching risk assessment, assigns low, medium, and high leaching potentials to GUS values of <1.8, 1.8-2.8, and >2.8 respectively. With the DT_{50} values listed above and respective K_{oc} values of 60 and 68.4 (Pesticide Properties Database 2018), CLO and TMX are assigned "high" GUS values of 5.43-6.98 and 1.84-4.25 respectively. No published work explains the high variability in published DT_{50} values (Goulson 2013) although it is likely due to the inherent properties (clay/sand/silt ratio, pH, etc.) of the soil (Bonmatin et al. 2015). Regardless, the continual and repeated use of these compounds has led to concerns over the role NSTs have in environmental loading and water contamination resultant from their high leaching risk (US-EPA 2010).

These leaching and runoff events have been suggested to follow biphasic (Gupta et al. 2008) as well as pulse (Thuyet et al. 2012) patterns. In the biphasic pattern, a precipitation event creates a large initial phase of neonicotinoid leaching and runoff that is comprised of largely unadsorbed neonicotinoid compounds. This is likely to occur in situations where precipitation events occur before maximum sorption to soil can occur. The second pattern is characterized by a slower rate of neonicotinoid loss associated with the gradual desorption of compound from soil. In a pulse runoff pattern, losses of applied product are directly proportional to the cumulative runoff depth for a given precipitation event (Thuyet et al. 2012). In both examples, concentrations of neonicotinoids in surface waters are predicted to be detectable at very low levels for a vast majority of the time but will spike in concentration with the occurrence of a precipitation event.

The concerns of environmental loading from NST use (US-EPA 2010) have since proven prescient. Reports of neonicotinoids in surface and ground waters have steadily increased over time with a few of these studies (DeLorenzo et al. 2012, Starner and Goh 2012, Sánchez-Bayo and

Hyne 2013, Hladik et al. 2014) suggesting contamination is the direct result of runoff or leaching. Furthermore, concentrations have exceeded freshwater invertebrate toxicity benchmarks (US-EPA 2016) on both acute (Anderson et al. 2013) and/or chronic scales (DeLorenzo et al. 2012, Starner and Goh 2012, Anderson et al. 2013, Sánchez-Bayo and Hyne 2013, Main et al. 2015). While often implicated, the direct role NST play in contributing to environmental contamination has only directly been studied in a few systems (Huseth and Groves 2014, Wettstein et al. 2016). Huseth and Groves (2014) reported a maximum TMX leachate concentration of ~10.7 ± 6.74 ng ml^{-1} recovered from a tension plate lysimeter in potato production with seed treatments however the initial seed treatment concentration was not reported or verified. Direct contamination of subsurface ground water was also reported with maximum concentrations of CLO and TMX at 0.225 and 0.580 ng ml^{-1} respectively (Huseth and Groves 2014). Wettstein et al. (2016) reported 4-month, flux averaged concentrations of 0.17 and 0.29 ng ml^{-1} for imidacloprid and TMX respectively from initial seed treatments of 0.45 mg imidacloprid and 0.30 mg TMX and seeding rates of 111,222 seeds ha^{-1}.

1.5 Non-target impacts of neonicotinoid use

1.5.1. In-field neonicotinoid non-target exposures

When compounds with high DT_{50} are used, such as the neonicotinoids, soil fauna within crop fields are likely to be exposed with a year round dose of insecticide, negatively impacting invertebrate abundance and diversity. As such, it is perhaps not surprising NST use has also been associated with a decrease in natural enemy abundance within numerous cropping systems according to a recent meta-analysis (Douglas and Tooker 2016). While a high abundance of natural enemies within a field does not guarantee successful management of pests to sub-economic levels, biological control of pests can only occur if a healthy population of natural enemies are present. This is particularly problematic in settings in which increased pest abundance is expected to be controlled by greater levels of natural control (predation, parasitism, disease) (Stinner and House 1990) as in fields in conservation tillage. Such ecosystem services are highly valuable to growers with one economic estimate valuing the control of native pests provided by natural enemies to be over $5.5 billion dollars in the US alone (Losey and Vaughan 2006). In one such instance, the use of NSTs in no till soybean fields suppressed the activity and density of slug predators, leading to a slug population outbreak and a yield decrease of 5% (Douglas et al. 2015).

1.6 Neonicotinoid non-target exposures beyond the planted field

1.6.1 Planter dust

The majority of maize planting in the US is achieved with pneumatic planters. The exhaust from these forced air planters contains abraded NST and contaminated fluency agents (talc, graphite, etc.), which can travel beyond 100 m. Given this rate of spread beyond the planted field, an Indiana study estimated that >94% of honey bee foragers are exposed to neonicotinoid insecticides in the form of residues deposited on flowers and/or aerosolized dust during maize sowing (Krupke et al. 2017). Instances of contaminated dust resulting in honey bee death has been reported in Italy (Bortolotti et al. 2009), Germany (Forster 2009, Pistorius et al. 2009), Slovenia (Van der Geest 2012), and Indiana (Krupke et al. 2012). While the risk to other non-target organisms has not been thoroughly quantified, deposition of contaminated dust on the surrounding landscape has been implicated as a stressor to monarch populations (Pecenka and Lundgren 2015) and other native pollinators (Rundlöf et al. 2015).

1.6.2 Within the soil

Evidence is increasing that NST can also negatively impact invertebrate communities within the soil. While very little has been reported on how NST impacts soil ecosystem function (Chagnon et al. 2015), two studies by Peck (2009a, 2009b) may provide some clues. Neonicotinoids were applied in turfgrass for scarab beetle control and the abundance of several nontarget groups measured. In comparison to untreated fields, a 54-62% reduction in abundance was reported for Collembola, Thysanoptera, and Coleopteran adults, over the course of two growing seasons. While not specifically investigated, Peck’s (2009b) conclusion discussed whether these reductions were ultimately harmful to ecosystem functions such as soil nutrient cycling and natural pest regulation and ultimately called for additional field relevant studies. These field realistic studies assessing the impact on ecosystem function are sparse due to the relatively recent wide scale use of neonicotinoids (Chagnon et al. 2015). As such, many of these questions have been addressed with short term laboratory studies. One group of particular concern and subsequent research is the earthworms (Pisa et al. 2015). While taxonomically distinct from Insecta, Oligochaeta also share neural pathways that neonicotinoids exploit (Elbert et al. 1991, Volkov et al. 2007). Exposure can occur through contact with contaminated soil, but also through ingestion of treated soil and plant litter (Kreutzweiser et al. 2009, Wang et al. 2012). One recent laboratory study looking at the effects of field relevant CLO concentrations (0, 1, 5, 10, 20, or 100 ppb) on *Lumbricus terrestris* reported significantly less food was consumed up to 8 weeks following introduction of contaminated food in the 20 and 100 ppb groups (Basley and Goulson 2017). Likewise, another study researching *Apporectodea* spp. found that a field-rate application of CLO impeded the decomposition of grass clippings for four months (Larson et al. 2012). In a conservation tillage system, earthworms are essential to the turnover of organic components of soil as well as creating macropores which benefit root growth and rain infiltration. While it appears that NST use can result in negative, non-target impacts on earthworms, little has been reported on the infield impacts of NST use. There is mounting evidence, however, that despite their targeted application only to crop seeds, NST active ingredients move well beyond the planted field both during and after planting.

1.6.3 Within the water

As mentioned, neonicotinoids have been reported at concentrations exceeding freshwater invertebrate toxicity benchmarks (US-EPA 2016) on both acute (Anderson et al. 2013) and/or chronic scales (DeLorenzo et al. 2012, Starner and Goh 2012, Anderson et al. 2013, Sánchez-Bayo and Hyne 2013, Main et al. 2015). The first large scale paper investigating neonicotinoid impacts with field data found large scale macroinvertebrate decline as a result of imidacloprid exposure (Van Dijk et al. 2013), although the authors did not account for co-occuring pesticides (Vijver and van den Brink 2014). Another correlative study found insectivorous bird decline was linked to increasing neonicotinoid use, not as the result of direct toxicity, but rather indirectly via effects on aquatic larval stages of their insect prey (Hallmann et al. 2014). These authors concluded that neonicotinoid contaminated water may not only negatively affect aquatic organisms, but have potentially wider trophic level impacts.

Mesocosm experiments have also been used to investigate the impacts of NST contamination of water. One such experiment found aquatic predator mortality increased by 52% when exposed to 352 ng CLO ml^{-1} over 21 d, in comparison to the 0.6 ng CLO ml^{-1} treatment

(Miles et al. 2017). While 352 ng CLO ml^{-1} is ~ 2 orders of magnitude greater that reported in literature, this study also demonstrated differential toxicity between aquatic herbivores and predators with the latter being more affected at lower concentrations. Another mesocosm experiment (Basley and Goulson 2018) investigated how neonicotinoid contamination impacted the colonization of water bodies. Several concentrations were used (CLO and TMX at 0.1, 1, 3, 7, 10, 15 ng ml^{-1}) and colonizing organisms were collected 38-d later and identified. The three most prominant colonizing groups were chironomids, ostracods, and *Culex*, of which the first two were most affected by increasing concentrations. Finally, a third mesocosm experiment utilized closed steams to investigate the impacts of a single dose of thiacloprid (0.1, 3.2, and 100 ng ml^{-1}) over the course of 7 months (Beketov et al. 2008). Twenty-one species were surveyed, of which 11 were considered long-lived (<1 generation per year) and were mostly comprised of representatives from Odonata, Plecoptera and Trichoptera. Ten short-lived (>1 generation per year) species were included and were comprised mainly of Diptera, Ephemeroptera, Isopoda, and Oligochaeta. The study reported differential recovery between short and long lived species with the latter taking up to 7 months to recover, and the former only taking 10 weeks at both the 3.2 and 100 ng thiacloprid ml^{-1} level. Overall, these mesocosm experiments, in concert with observational and correlative field studies, have allowed researchers to better describe and predict how neonicotinoid contamination of water bodies can lead to nontarget impacts in both aquatic and terrestrial environments.

1.7 Purpose of this dissertation

It is the overarching goal of this dissertation to better understand and describe how NST use moves into and affects the wider environment. Unconsidered and understudied pathways exist for neonicotinoids to impact non-target species. For example, considering the numerous instances of runoff and leaching of neonicotinoids, relatively little research has been conducted or reported on the environmental fate of neonicotinoids (Goulson 2013). This is particularly true of clothianidin, the most widely-used seed treatment in North America. At the conclusion of this dissertation, I will develop a greater understanding of the contribution of maize NST to environmental contamination and provide a basis upon which future research directions can be built as well as define key problem areas.

The first step in achieving this was to quantify the amount of CLO translocated into target plant tissue (Chapter 2) (Fig. 1). Doing so allowed me to estimate the pest management window of this approach through the comparison of CLO concentrations within treated versus untreated plants. The pest management potential of NST was considered finalized upon statistical similarity of CLO concentrations within treated versus untreated plants as the growing season progressed. Prior to this paper, the pest management window had not been defined for NST and provided a possible explanation of inconsistent yield results reported above (Section 1.3), which was further explored in Chapter 3 within the confines of a meta-analytical framework using Indiana insecticide efficacy trail data from 2000-2015. Furthermore, by determining what portion of a NST makes it into the plant, I was by extension able to estimate what portion of the CLO ST that was lost to the environment. This was further explored in Chapter 4 through the use of field lysimeters which allowed me to quantify the magnitude and timing of CLO concentrations in leachate from tile drain fields. Finally, manipulative laboratory experiments were conducted to determine the uptake dynamics of aqueous CLO, a proxy for agricultural runoff and leachate, in the aquatic macrophyte, gibbous duckweed (*Lemna gibba*) and bioassays utilizing CLO-contaminated duckweed were conducted on a duckweed-associated insect (*Rhopalosiphum nymphaeae*, waterlily aphid (Linnaeus)) to investigate potential impacts on higher trophic levels.

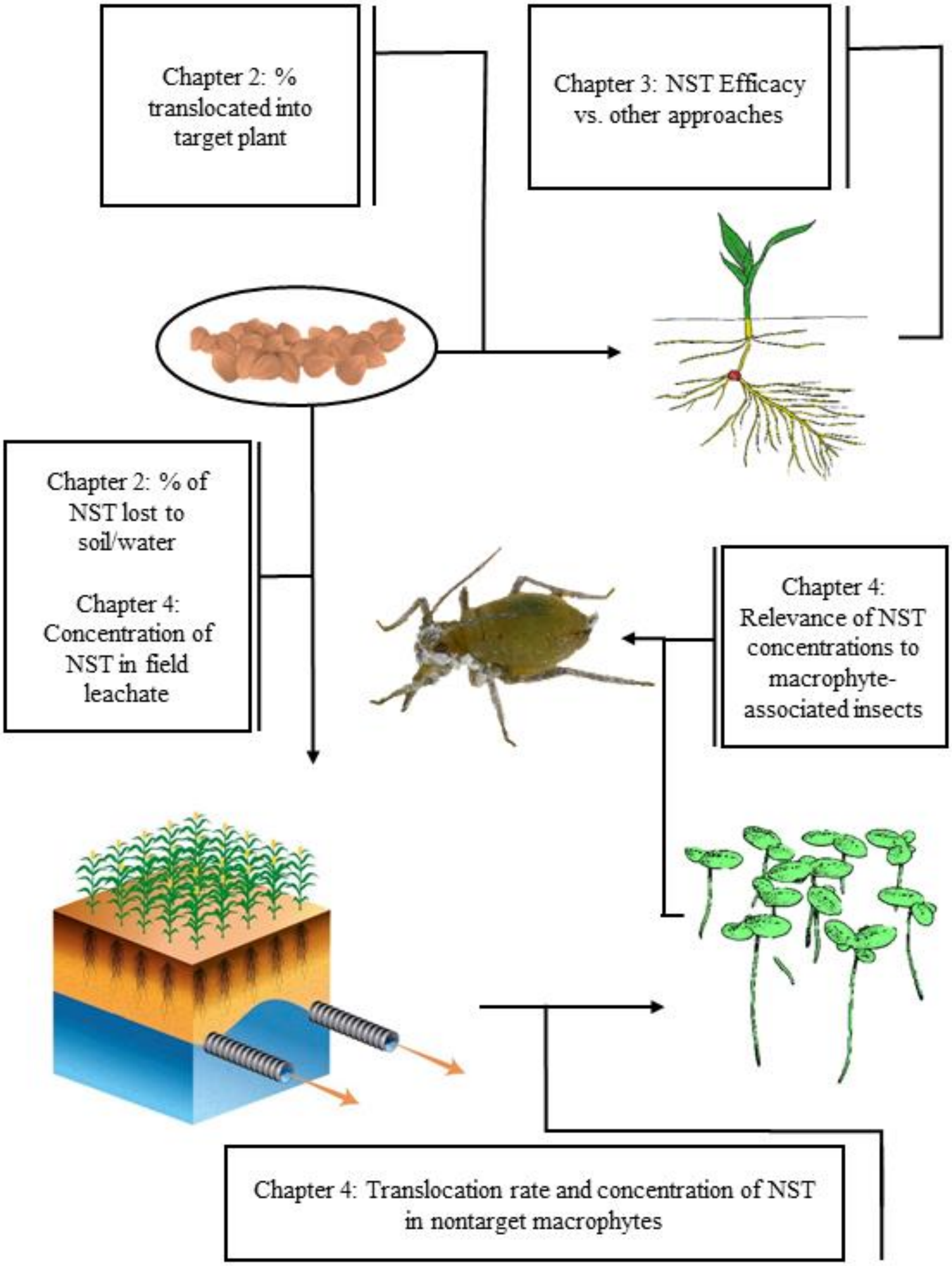


Figure 1. Flowchart of dissertation objectives.

CHAPTER 2: TRANSLOCATION OF THE NEONICOTINOID SEED TREATMENT CLOTHIANIDIN IN MAIZE

2.1 Introduction

The neonicotinoids are a relatively new group of systemic insecticides. The first commercially available compound, imidacloprid (Bayer CropScience), was available in the early 1990s, with other compounds following in the 2000s. They have since become the most widely used insecticide class worldwide (Jeschke et. al. 2011, Pollack 2011). Their rapid and widespread adoption has been attributed to low mammalian toxicity, systemic and translaminar properties, lack of resistance upon market entry, increasing restrictions and regulations on older pesticide groups, and potential for a wide variety of application methods (Elbert et al. 2008). Neonicotinoids are most frequently used as seed treatments (ST), comprising 80% of the ST market worldwide in 2008 (Jeschke et al. 2011). Maize (corn), along with the other three major US field crops (soybean, wheat, and cotton) by area planted (USDA-NASS 2014), all have neonicotinoid seed treatment (NST) registrations using the active ingredients (AI) imidacloprid, clothianidin (CLO) (Bayer CropScience), and thiamethoxam (Syngenta Crop Protection) (Douglas and Tooker 2015).

The neonicotinoids are used in US maize production solely as STs, with >80% of maize planted annually being treated with either CLO or thiamethoxam at application rates of 0.25-1.25 mg kernel^{-1} prior to sale to the grower (Krupke et al. 2012, Douglas and Tooker 2015). While Bt hybrids largely control damage from the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Storer et al. 2006, Ma et al. 2009), the primary maize pest of Indiana (Hokkanen 1991) (recent resistance notwithstanding (Gassman et al. 2011)), the 1.25 mg kernel^{-1} rate is also labeled to control the larval stage of this pest (Cook and Steffey 2004, DeKalb 2016). NSTs are also labeled to control a range of other secondary, early season root and seed pests including wireworms (Riley and Keaster 1979), seedcorn maggots (Higley and Pedigo 1984), and white grubs (Jordan et al. 2012). White grubs preferentially attack the root tissue of young seedlings (Metcalf and Metcalf 1993) and wireworms occasionally burrow into the stems of young seedlings (Royer et al. 2004) however, both will readily feed on the germinating seed (Youngman et al. 1993), the primary site of seedcorn maggot attack (Higley and Pedigo 1984). While seedcorn maggot injury pressure can be reliably predicted based upon incorporation of a green cover crop into the soil (Hammond

1990), economic infestations of these secondary pests are typically sporadic and difficult to predict (Royer et al. 2004). This has in part, led to the widespread adoption of NSTs as a prophylactic measure of minimizing pest damage risk.

Both CLO and thiamethoxam are hydrolytically stable with relatively high respective solubilities of 0.327 g L^{-1} at 20˚C and 4.1 g L^{-1} at 25˚C; these solubilities confer systemic properties (Jeschke and Nauen 2008). Despite widespread use in maize systems, little has been published on the translocation efficiency of the NST delivery method in maize, or the distribution and concentration of these compounds throughout the plant once material is translocated (Goulson 2013). A potted plant study (Myresiotis et al. 2015) found thiamethoxam shoot concentrations in maize to be 0.62 µg thiamethoxam g^{-1} 21 days post plant (DPP), with a gradual concentration decrease to 0.13 µg thiamethoxam g^{-1} at 36 DPP from an initial 0.1 mg thiamethoxam per kernel ST. Root tissue thiamethoxam was not quantified nor ST efficacy, viewed in terms of the percentage of initial ST translocated to plant tissue. Furthermore it is unknown what concentrations would provide a pest management benefit. To inform the debate surrounding the costs and benefits of this management approach, these data represent key parameters in defining a "pest management window" where these products could be expected to provide crop protection, as well as informing environmental fate studies once the AI is liberated from the seed.

Previously published greenhouse studies examining imidacloprid ST in maize report 20% of the AI being translocated into the plant with the remaining 80% assumed to enter the soil matrix and/or leach away from the plant and its root zone (Sur and Stork 2003). Once applied and within soil, the time required to dissipate 50% of the applied AI (DT_{50}) is highly variable within and between neonicotinoid compounds. The DT_{50} of imidacloprid, thiamethoxam, and CLO, the three compounds used in NSTs (Elbert et al. 2008), are 40 (Rouchaud et al. 1994) to 270 (Bonmatin et al. 2005), 7.1 to 92.3 (Hilton et al. 2016), and 277 to 1386 days (US-EPA 2010), respectively, under field conditions. There is no published work explaining the high variability in published DT_{50} values (Goulson 2013).

Given their soil persistence and repeated use, concern exists over the potential of neonicotinoids in environmental loading and water contamination via leaching and field runoff (US-EPA 2010). While not the only metric used to assess leaching risk, the Groundwater Ubiquity Score (GUS) (Gustafson 1989) relates the compound's soil organic carbon-water partitioning coefficient (K_{oc}), and DT_{50} and assigns high, medium, and low leaching potentials to respective

GUS values of >2.8, 1.8-2.8, and <1.8. Both CLO and thiamethoxam have GUS values of 5.43-6.98 and 1.84-4.25 each, based upon respective K_{oc} values of 60 and 68.4 (Pesticide Properties Database 2017.) and DT_{50} values listed above.

Increasing detections of neonicotinoids in a range of surface and ground waters have been reported. A few of these studies (DeLorenzo et al. 2012, Starner and Goh 2012, Sánchez-Bayo and Hyne 2013, Hladik et al. 2014) suggest water contamination as the direct result of runoff or leaching and in multiple instances, concentrations exceeded either chronic (DeLorenzo et al. 2012, Starner and Goh 2012, Anderson et al. 2013, Sánchez-Bayo and Hyne 2013, Main et al. 2015) or acute (Anderson et al. 2013) toxicity benchmarks for freshwater invertebrates (US-EPA 2016). Detection of neonicotinoids in non-target vegetation has also been attributed to lateral subsurface movement in the USA (Long and Krupke 2016), the UK (Botías et al. 2015), and implicated as a pathway for neonicotinoid contamination of organic fields (Mogren and Lundgren 2016). Although little has been published quantifying the ultimate impact of these compounds in the environment, correlative studies have indicated that these compounds may be causal agents of long-term macroinvertebrate decline in surface water (Van Dijk et al. 2013), (although this hypothesis has been disputed as not accounting for the presence of other insecticides (Vijver and van den Brink 2014) and of insectivorous bird populations (Hallman et al. 2014).

The numerous examples of environmental detections of neonicotinoids coupled with a variable soil half-life highlight the potential of these compounds to accumulate in the environment. However, the mechanism is unclear. A key unknown in untangling these mechanisms is the amount of material that enters the target (i.e. crop plants). My goal was to quantify these levels in space (various plant regions) and time (across the early growing season) in order to: 1) define the pest management window afforded by these compounds and 2) determine one component of the environmental fate of the NST. The work described here provides baseline information on the translocation of the major NST of maize, CLO, into various regions of the growing plant, using field-collected plants beginning at seed sowing and continuing through the growing season.

2.2 Materials and methods

2.2.1 2014 & 2015 field site and experimental design

Planting of hybrids DeKalb 6179 (2014) and Spectrum 6241 (2015) took place on May 5th at the Throckmorton Purdue Agricultural Center (40°18'00.7"N 86°43'37.0"W). The 5-year precipitation average in May ($\bar{X}$ ± SD) for this site is 67.2 ± 27.96 mm and the soil is characterized as loam with a 43.6/38.4/18 sand/silt/clay ratio. Four treatment levels were evaluated: untreated seed ("Naked"), in which no ST was applied, a fungicide only ST ("Fungicide"), a low rate applied at 0.25 mg CLO kernel^{-1} ("Low"), and a high rate of 1.25 mg CLO kernel^{-1} ("High"). The "Fungicide", "Low" and "High" treatments were also treated with the fungicides metalaxyl, trifloxystrobin, and ipconazole at respective rates of 0.92, 4.79, and 2.4 g/100 kg of seed. Each treatment level was replicated four times in a randomized complete block design with treatment plots measuring 3.05 x 36.58 m in 2014 and 3.05 x 33.53 m in 2015. The previous crop in both years was a "trap crop" of late planted maize to maximize western corn rootworm egg deposition. The late season "trap crop" was comprised of corn hybrids expressing Bt genes targeting lepidopteran pests and treated with the "Low" CLO rate. Given the instances of subsurface flow (Botías et al. 2015, Long and Krupke 2016, Mogren and Lundgren 2016), and proximity of untreated plots to treated plots (plots ~3m wide), CLO contamination of untreated plots was expected and inevitable - a truly neonicotinoid-free field is not achievable in the maize and soybean production areas of North America (Douglas and Tooker 2015). Attempts were made to minimize contamination by only collecting samples from the 2 central rows of each plot.

2.2.2 2014 & 2015 sampling, root ratings, stand and yield

Sampling consisted of removing ten randomly selected maize plants intact from each plot and storing them at -20˚C for later processing. Five of these ten samples were processed with a modified QuECHeRs protocol (Anastassides and Lehotay 2003) (details in Appendix A) with the remaining five serving as reserve samples. At 21 days post planting (DPP), plant tissue CLO concentrations were expected to approach zero based upon preliminary data gathered in 2013, so sampling was reduced to five plants per treatment plot every other week with three of the five samples being processed and analyzed. Sampling was concluded at 61 DPP in both years as CLO concentrations in treated plants were expected to approach the CLO concentrations of untreated plants by this time based upon preliminary data. Increases of in-plant CLO concentrations were not expected either. Stand counts were conducted at the V2/V3 stage on June 3rd and 8th in 2014 and 2015 respectively to assess seed germination. Root damage by the western corn rootworm was scored (Oleson et al. 2005) on July 18th and the 23rd in 2014 and 2015 respectively. An average treatment plot root rating was calculated from four roots in 2014 and five roots in 2015. Maize was harvested and yield calculated on October 10th in 2014 and October 15th in 2015 after adjusting maize moisture to 15.5% (Brunson 1959).

2.2.3 Calculation of economic damage

The minimum node-injury required to cause economic damage was calculated for both sampling years using the Oleson et al. (2005) method. Calculations included a range of insect control costs (\$17.5-\$55 ha^{-1}), assumed a moderate level of environmental stress (21.7 heat stress degree-days) and used an average of \$14.96/100 kg for market value given the similarity of Indiana maize marketing values between 2014 and 2015 (\$14.76 and \$15.15/100 kg respectively) (USDA-NASS 2017a).

2.2.4 Sample preparation for chemical analysis

A modified QuECHeRs protocol (Anastassides and Lehotay 2003) (details in Appendix A) was used to prepare samples for chemical analysis in both years. Individual samples were split into root, seed, and shoot regions in both years (Fig. 2) after residual soil was removed from plant tissue by running the sample underneath a gently running faucet. For samples weighing <1 g per plant region, the root region was considered the radicle and seminal roots, while the shoot region was defined as all plant tissue from the base of the mesocotyl to the stem apex. For samples weighing >1 g per plant region (samples collected after 20 and 16 DPP in 2014 and 2015, respectively), subsections of the stem apex, the area of newest growth, and of a randomly selected root were used for homogenization and further analysis of shoot and root regions, respectively. No more than 1 g of plant tissue was used per tissue region due to space limitations of homogenization tubes. Root and shoot regions for a given sample were scored as “complete” (>80% present) or “incomplete” (<80% present) prior to homogenization. An average % AI translocated for each plant region (root and shoot) was calculated from these data. If both the corresponding root and shoot region of a given plant was scored as “complete”, the plant was scored as a “total sample” and the respective concentrations for both regions were combined to calculate an average overall % AI translocation per plant.

2.2.5 Determination of pest activity period

The active period of western corn rootworm, seedcorn maggot, wireworm, and white grubs was estimated in both years to compare it to the NST protection window. For western corn rootworm, this was accomplished by checking maize roots daily in a nearby field until observation of neonate western corn rootworm larvae. The seedcorn maggot, wireworm, and white grubs were not directly monitored as they were not present in our study field, and economic infestations are typically sporadic and unpredictable (Royer et al. 2004). Egg hatch for seedcorn maggot was estimated with a degree day model (Sanborn et al. 1982) used in conjunction with atmospheric data (Indiana State Climate Office 2016). Adult seedcorn maggot emergence and subsequent oviposition occurs in early May for central Illinois (Metcalf and Metcalf 1993), so calculations assumed adult emergence occurred on May 1st for Indiana given the similarity in latitudes. Degree-day models were inappropriate to define an active period for wireworm and white grubs due to their multispecies status so peer-reviewed and extension literature was searched instead (Purdue University Agricultural Communication 2015).

2.3 Statistical analyses

2.3.1 Effects of NST on yield, stand count, and root ratings

Yield, stand count, and root ratings were analyzed using SAS PROC MIXED (SAS University Edition 2015) in both years. Treatment and block was included as fixed effects in all six models. A Tukey (HSD) post-hoc analysis was used to separate which treatment means were significantly different from each other ($\alpha < 0.05$) following a significant test result ($P < 0.05$) (Gotelli and Ellison 2004).

2.3.2 Determination of protection window

Two different approaches were used to estimate the pest management window. The first approach fit a first order decay function (Nose 1987) using a Levenberg-Marquardt nonlinear least-squares algorithm with the package minpack.lm in the R statistical language (Elzhov et al. 2016, R Core Team 2016) to translocation data for each plant region (root, seed, and shoot) as a function of DPP. Decay curves were visually examined to estimate at what DPP the rate of change decreased to where concentration appeared to “flatten out”. Protection was considered lost at this point.

The second, more conservative approach, analyzed translocation data using a multivariate approach to repeated measures with SAS PROC GLM (SAS University Edition 2015). Prior to analysis, data were natural log transformed to conform to normality assumptions and confirmed with visual inspection of residuals. Separate models were used for each plant region (root, seed, shoot) in both sampling years (2014, 2015). Fixed main effects included treatment, block, sampling date, and a multivariate treatment*sampling date interaction effect as predictors of CLO concentration. Univariate results of the repeated measures ANOVA were analyzed in concert with visual inspection of decay functions to inform designation of appropriate linear contrasts ($\alpha = 0.05$) for determining when the residue curves converged in time. When CLO concentration in treated plants was similar to the untreated controls, any protection afforded by the NST was considered expired. Two sets of contrasts were made: “Naked” + “Fungicide” vs “Low” and “Naked”+”Fungicide” vs “High”.

2.4 Results

2.4.1 Sampling and effects of NST on yield, stand count, and root ratings

In 2014, sampling and AI extraction was carried out at 6, 8, 10, 13, 15, 17, 20, and 34 DPP and at 5, 7, 9, 12, 14, 16, 19, 47, and 61 DPP in 2015. Freezer failure resulted in no AI extraction past 34 DPP in 2014 samples and resulted in the loss of 33 DPP samples in 2015. The seed region was only recovered up to 20 and 19 DPP in 2014 and 2015 respectively. The limit of CLO detection was determined to be 0.1 ng g^{-1}.

In 2014, neither treatment or block had a significant effect on root ratings ($F_{3,9} = 0.98$, $P = 0.4431$; $F_{3,9} = 0.83$, $P = 0.5097$). For yield, only block had a significant impact ($F_{3,9} = 13.65$, $P = 0.001$) whereas treatment did not ($F_{3,9} = 1.06$, $P = 0.4131$). Neither variable had a significant effect (Treatment: $F_{3,9} = 0.50$, $P = 0.6937$; Block: $F_{3,9} = 2.73$, $P = 0.1063$) on stand count (Table 1).

In 2015, neither treatment ($F_{3,9}$ = 1.57, *P* = 0.2639) or block ($F_{3,9}$ = 3.44, *P* = 0.0654) had a significant effect on root ratings, stand (Treatment: $F_{3,9}$ = 2.57, *P* = 0.1193; Block: $F_{3,9}$ = 0.93, *P* = 0.4646), or yield (Treatment: $F_{3,7}$ = 0.83, *P* = 0.5192; Block: $F_{3,7}$ = 1.78, *P* = 0.2392) (Table 1).

Table.1. Means of yield in kg ha^{-1}, plants per hectare (PPH) and root ratings (RR) for both 2014 and 2015 field season. Within a given column and year, means followed by the same letter denote statistical similarity as determined by Tukey HSD comparisons at *P* = 0.05

2014	**Yield±SE**	**n**	**PPH±SE**	**n**	**RR±SE**	**n**
Naked	13216±547 a	4	201133±34779 a	4	0.006±0.004 a	4
Fungicide	13741±618 a	4	191870±4360 a	4	0.031±0.012 a	4
Low	13997±523 a	4	220427±19642 a	4	0.019±0.008 a	4
High	13743±859 a	4	195155±15643 a	4	0.028±0.017 a	4
2015						
Naked	13159±552 a	4	202140±9275 a	4	0.193±0.059 a	4
Fungicide	12072±890 a	3	202140±3914 a	4	0.456±0.259 a	4
Low	13423±501 a	3	184183±6637 a	4	0.378±0.166 a	4
High	13266±184 a	4	207458±3602 a	4	0.128±0.006 a	4

2.4.2 Calculation of economic damage

The minimum node-injury required to cause economic damage in both sampling years ranged from 0.245-0.771 with a respective control cost of \$17.5-55 ha^{-1}. Economic injury levels were only reached in 2015 in the “Low” and “Fungicide” treatment plots with a control cost < \$27 ha^{-1} and < \$32.5 ha^{-1} respectively (Table 1 and Fig. 3).

2.4.3 Translocation efficacy

For all plant regions, a greater proportion of the initial CLO seed treatment was successfully translocated in 2015 plants (Fig. 4). A maximum of 0.26 and 1.18% of the initial “Low” treatment rate was translocated to respective root and shoot tissues in 2015 whereas a maximum translocation of 0.20 and 0.65% was recovered in the root and shoot region with the

"High" treatment. In all instances, less than 1.5% of the initial ST was translocated to the root and shoot region in "total sample" homogenizations. The "High" treatment rate experienced a greater proportion of the initial ST translocation in 2014 but not in 2015, where the "Low" application rate resulted in greater overall translocation.

2.4.4 Determination of pest activity period

The first western corn rootworm neonate larvae of the season were observed at 27 and 28 DPP in 2014 and 2015, respectively (Fig. 5). Larval emergence from overwintered eggs typically occurs during late May to early June in this region of Indiana (Bledsoe and Obermeyer 2010). Our seedcorn maggot model predicted egg hatch at planting and one day before in 2014 and 2015, with larval development (the damaging stage) being completed at 16 DPP and 12 DPP for 2014 and 2015, respectively.

No defined "attack period" for our study region is reported in peer-reviewed literature for white grubs and wireworm beyond that of a generalized early season root and seed feeding pest (Royer et al. 2004), however extension literature (Purdue University Agricultural Communication 2015) places white grub and wireworm attack from mid-April to mid and late June respectively in Indiana; these estimates are necessarily variable as they depend largely upon degree day accumulations. For the purpose of this project, the end of white grub and wireworm attack was considered June 15th (41 DPP) and June 30th (56 DPP) respectively.

2.4.5 Determination of protection window

The exponential decay equation explained 34.4 to 46.1% and 54.1 to 86% of the variance in tissue-bound AI concentration for the respective root and shoot regions of treated plants. In comparison to NST plants in both years, "Naked" plants had lower R^2 values indicating a smaller proportion of explained variance resultant from the DPP predictor and exponential fit (Table 2). Root and shoot regions had larger R^2 values than their corresponding seed region indicating tissue-bound AI concentration better conformed to the exponential decay prediction. This was characterized in the root region by a flattening out of the decay curve around 17 and 20 DPP for the "Low" treatment and 15 and 25 DPP for the "High" treatment in 2014 and 2015 respectively (Figs 5 and 6). By taking the average of both years for each treatment type, the protection window in the root region for "Low" and "High" treatments was considered to be 18.5 DPP and 20 DPP respectively. For the shoot region, the flattening out of the decay curve occurred around 17 and 22 DPP for the "Low" treatment and 20 and 33 DPP for the "High" treatment in 2014 and 2015 respectively. The decay curve was not used to estimate a protection window for the seed region given its overall poor fit to the data.

Table 2. R^2 values describing the fit of the translocation data as a function of days post planting (DPP) for clothianidin as estimated by the exponential decay function: $C = C_0e^{-kt}$

Region	**Treatment**	**2014**	**2015**
Root	Naked	0.101	0.304
	Fung	0.499	0.618
	Low	0.461	0.344
	High	0.421	0.437
Seed	Naked	0.272	0.214
	Fung	0.287	0.400
	Low	0.494	0.041
	High	0.244	0.027
Shoot	Naked	0.351	0.344
	Fung	0.523	0.681
	Low	0.585	0.731
	High	0.541	0.86

A significant multivariate treatment*sampling date interaction was recorded once in the Root 2014 model (Table 3) indicating relative treatment differences of in-plant CLO concentrations were similar across the sampling period in the remaining models. As the univariate treatment effects in all models remained highly significant ($P < 0.001$) until 20 DPP (Table 4), only contrasts taking place on or after 15 DPP were considered. Visual inspection of decay curves confirmed this initial assessment and further reduced the number of *a priori* contrasts made.

Table 3. F-values and estimated degrees of freedom (df) for the multivariate repeated-measures ANOVA model describing in-plant concentrations of clothianidin over the sampling period in 2014 and 2015 for the three plant regions (Root, Seed, Shoot). Significant results are denoted by an * with $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$

Region	2014 Factor	df	F-value	2015 Factor	df	F-value
Root	Time	7,3	418.22^{***}	Time	8,1	391.84^{*}
	Treatment*Time	21,9.17	4.64^{*}	Treatment*Time	24,3.50	4.43
Seed	Time	6,4	314.37^{***}	Time	6,3	14.05^{*}
	Treatment*Time	18,11.80	1.15	Treatment*Time	18,8.97	2.42
Shoot	Time	7,3	181.26^{***}	Time	8,1	328.26^{*}
	Treatment*Time	21,9.17	2.27	Treatment*Time	24,3.50	1.25

In 2014, concentrations were similar or converged ($P > 0.05$) at 17, 20, and 34 DPP for "Naked"+"Fungicide" vs "Low" contrasts for the respective root, seed, and shoot tissues, but not for the "Naked"+"Fungicide" vs "High" contrasts which remained different throughout the sampling period (Table 5), with the exception of the shoot region at 34 DPP. In 2015, this did not occur for the root or shoot region until 47 DPP for either contrast set. It is possible that concentrations converged at an earlier period however freezer malfunction resulted in the loss of 33 DPP samples.

To compare pest phenology to concentration data, the shoot region was considered protected until 34 DPP regardless of CLO application rate and treated seed was considered protected for the entirety of the seed recovery period (up to 20 DPP in both years) despite concentration convergence at 17 DPP in the "Naked"+"Fungicide" vs "Low" contrasts (Table 5). The root region was considered protected up to 34 and 47 DPP for the respective "Low" and "High" treatment rates. Protection was considered to last up to 34 DPP for the "Low" treatment rate as a compromise between 2014 and 2015 data. Root protection was first lost at 17 DPP in 2014 but was still provided at 19 DPP in 2015. As a freezer malfunction resulted in the loss of 33 DPP samples in 2015, the next possible sampling date was 47 DPP in which protection had already been lost. It is unknown whether protection had been lost by 33 DPP in 2015, however by selecting 34 DPP as the date of protection loss, I balance the possibility of underestimating the protection window based on 2014 data and overestimating the protection window based on 2015 data.

Table 4. Univariate F-values and degrees of freedom (df) generated following the multivariate repeated-measures ANOVA model describing influence of initial clothianidin seed treatment on in-plant concentrations of clothianidin over the course of multiple days post planting (DPP) in 2014 and 2015 and three plant regions (Root, Seed, Shoot). Significant results are denoted by an * with $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

2014				**2015**			
DPP	**Root**	**Seed**	**Shoot**	**DPP**	**Root**	**Seed**	**Shoot**
6	142.15***	71.67***	40.78***	**5**	52.89***	424.34***	429.43***
8	79.27***	112***	121.111***	**7**	24.97***	369.71***	79.38***
10	32.59***	63.74***	116.15***	**9**	321.89***	715.71***	229.14***
13	67.51***	84.58***	64.44***	**12**	50.81***	128.6***	37.17***
15	177.66***	143.84***	108.55***	**14**	149.45***	82.35***	101.01***
17	21.51***	52.19***	91.78***	**16**	71.75***	93.66***	93.13
20	17.2***	24.08***	37.15***	**19**	62.14***	44.11***	87.86***
34	3.69	0.244	0.027	**47**	2.34		1.87
				61	2.77		0.19
df	**3,9**	**3,9**	**3,9**	**df**	**3,8**	**3,8**	**3,8**

2.5 Discussion

These results are the first to use field experiments to quantify in-plant concentrations of CLO, the principal neonicotinoid AI currently used in North American maize production, and demonstrate a rapid reduction in tissue-bound CLO beginning in the days following seed sowing. An exponential decay equation (Nose 1987) provides explanatory power in describing the relationship between CLO concentrations solely as a function of time in the root and shoot region of treated plants. Given the water solubility of CLO (0.327 g L^{-1} at 20˚C; Jeschke and Nauen 2008), and the low percent of AI remaining on the seed at the first sampling date, it is likely the removal of CLO from the seed surface was rapid following seed sowing and followed an exponential decay pattern; our sampling protocol may have been initiated too late (5 and 6 DPP in 2015 and 2014, respectively) to observe this trend fully. This may explain why the seed region in all treatments had the lowest R^2 values associated with the exponential decay equation (Table 2). Furthermore, the combination of CLO's high water solubility and long soil half-life (277-1386 days (US-EPA 2010)) is likely underlying our observations of CLO in untreated plant tissues, either as a result of lateral movement between plots and/or as a residue from the previous planting season. This is not unexpected as expression of neonicotinoids in non-target plants has previously been attributed to subsurface movement (Botías et al. 2015, Long and Krupke 2016).

Table 5. F-values of *a priori* contrasts comparing untreated maize seed (Fungicide + Naked) to 0.25 mg clothianidin kernel^{-1} (Low) and 1.25 mg clothianidin kernel^{-1} (High) at various days post planting (DPP) for three different plant regions (Root, Seed, Shoot) in 2014 and 2015. Significant results are denoted by an * with $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

		2014		**2015**	
Region	**Contrast**	**DPP**	**F-value**	**DPP**	**F-value**
Root	Fungicide+Naked vs Low	15	$F_{1,9}=38.86^{***}$	19	$F_{1,8}=57.11^{***}$
		17	$F_{1,9}=1.89$	47	$F_{1,8}=5.11$
		20	$F_{1,9}=0.66$	61	$F_{1,8}=2.81$
	Fungicide+Naked vs High	17	$F_{1,9}=57.59^{***}$	19	$F_{1,8}=180.27^{***}$
		20	$F_{1,9}=47.08^{***}$	47	$F_{1,8}=4.09$
		34	$F_{1,9}=10.13^{*}$	61	$F_{1,8}=1.41$
Seed	Fungicide+Naked vs Low	17	$F_{1,9}=18.98^{**}$	19	$F_{1,8}=53.89^{***}$
		20	$F_{1,9}=3.97$		
	Fungicide+Naked vs High	17	$F_{1,9}=155.55^{***}$	19	$F_{1,8}=110.31^{***}$
		20	$F_{1,9}=69.20^{***}$		
Shoot	Fungicide+Naked vs Low	17	$F_{1,9}=26.69^{***}$	19	$F_{1,8}=89.05^{***}$
		20	$F_{1,9}=8.17^{*}$	47	$F_{1,8}=0.38$
		34	$F_{1,9}=0.56$		
	Fungicide+Naked vs High	20	$F_{1,9}=107.03^{***}$	19	$F_{1,8}=242.76^{***}$
		34	$F_{1,9}=4.47$	47	$F_{1,8}=4.77$

These data also provide a potential mechanism to explain a range of field observations from previously published literature. Numerous studies have reported inconsistent yield benefits of NSTs in maize, including finding no advantages of the NST approach when compared to maize seed having no insecticide applied (Cox et al. 2007, Wilde et al. 2012, Jordan et al. 2012). Similarly, our study found no statistical differences in stand count, root ratings, or yield between treated and untreated seed in both years. While the presence of root feeding pests was documented in both years as evidenced by root ratings (Table 1), economic injury was only observed in the 2015 “Low” and “Fungicide” treatments and only if NST cost was assumed to be <\$27 ha^{-1} and

<\$32.5 ha^{-1} respectively. In other words, no economic benefit would be realized if the respective application costs were >\$27 ha^{-1} and >\$32 ha^{-1} for the "Low" and "Fungicide" treatments. This interaction between insect damage, crop yield, and insecticide cost is expressed in the following equation (Pedigo and Rice 2009):

$$EIL = C/V*b*K$$

Where the economic injury level (*EIL*) is defined as management cost per area (*C*) over market value per produce unit (*V*) multiplied by yield loss per insect (*b*) and the proportionate reduction in potential injury (*K*).

Out of necessity, I assumed a range of insect control costs (\$17.5-\$55 ha^{-1}) for *C* because the actual cost is not disclosed to growers or available in the published literature. Estimates range widely from between ~17.5 ha^{-1} (Studebaker 2007, Myers and Hill 2014) to \$37.5 ha^{-1} (Seagraves and Lundgren 2012). Below, I outline two sets of calculations that shed light on the potential fit for this pest management approach.

Using a value of *K* of 1 (i.e. 100% reduction in pest injury) is admittedly unrealistic and unattainable, but provides a best case option for exploring different scenarios given market values and insect control costs influence *EIL* calculation. For example, transposing our observed 2015 damage results and using the recent 5-year high for Indiana maize market values of \$28.46/100 kg in 2012 (USDA-NASS 2016a), I would expect economic injury in the 2015 "Naked", "Low" and "Fungicide" treatments with respective control costs <\$26, <\$51, and <\$62 ha^{-1} (Table 1 and Fig. 3). However a more informative approach for growers is to use the 2014-15 average (\$14.96/100 kg), which coincides with the 2016 mean market values (\$14.87/100 kg (USDA-NASS 2017a)) to calculate the *EIL*; values are approximately 50% lower than 2012 values resulting in control costs of \$27 ha^{-1} and \$32.5 ha^{-1} for respective "Low" and "Fungicide" treatments. While the 2012 example demonstrates spending an initial ~\$17.50 ha^{-1} on NST may be justified under high market prices or high pest pressure, lower market prices (2014-16) limit any additional control tactics a grower can afford.

The results of previous reports (Cox et al. 2007, Wilde et al. 2012, Jordan et al. 2012) and the findings I report here suggest that *K* is lower than 1. Comparison of damage in the untreated versus treated plants allows us to estimate *K*. In 2015, the average root rating for the "Naked" and "Fungicide" treatments was 0.3245 and the "High" was 0.128 leading to an estimated *K* value of 0.395. Using this value, none of the tested treatments reached economic injury as the lowest tested treatment cost (17.50 ha^{-1}) would require a minimum root rating of 0.53 to reach economic injury levels. A less efficacious pest management approach effectively raises the economic threshold.

Our economic threshold estimates using these data provide a starting point for discussion, but the overall benefit of NSTs cannot be fully assessed without knowledge of the actual cost of NSTs for maize growers. Maize seed without NSTs is increasingly scarce in the current marketplace (Douglas and Tooker 2015). A true “free market” approach to seed availability, including a wide selection of readily available NST-free maize seed would provide a basis for cost comparison while allowing producers, consultants and researchers to readily make on-farm comparisons and determine if and when NST costs are justified.

In assessing potential benefits of this approach, I chose to use in-plant CLO concentrations to construct a pest management window for maize plants grown from treated seeds. Planned contrasts provided a highly conservative estimate of the date after planting at which the CLO concentration in treated plant tissue was statistically similar to that of untreated plant tissue. This approach to the development of a pest management window is highly conservative, in that it assumes that even very low levels of the AI in plant tissue is likely to provide pest management benefit, a notion that has not been tested empirically in the lab or field. This is the most parsimonious initial approach for interpreting these data from a pest management standpoint because oral LD_{50} concentration data for NSTs have not been reported for the target pest insects.

This study also demonstrates that when deploying NSTs, consideration should be given to pest biology, more specifically to how the pest’s activity window and region of attack overlap periods where a pest management benefit of NSTs could be expected. In the case of the key pest of maize, western corn rootworm, the damaging larval stage was active starting at 27 and 28 DPP in 2014 and 2015 respectively (Fig. 5). As *a priori* contrasts suggested CLO STs at the “High” rootworm rate (Cook and Steffey 2004, DeKalb 2016) stopped providing protection to the root tissue by 47 DPP, ~20 days of western corn rootworm protection were provided (Table 5). This may be a sufficient window and insecticide concentration level (from 31.47 µg g^{-1} (6 DPP) to 0.02 mug g^{-1} (47 DPP) for “High” root tissue in 2015) to deter or kill neonate western corn rootworm larvae, although no data exist to support or refute this hypothesis. Alternatively, decay curve analysis shows the likely root protection window extended to 18.5 and 20 DPP for the respective “Low” and “High” treatments, ending well before western corn rootworm emergence and presenting a poor fit with the phenology of this key pest. While it is possible that the ambient AI concentration in soil around roots is high enough to provide control, this hypothesis has not been tested experimentally.

Finally, the rapid decrease in concentration of insecticide within plant tissues points to a broader question for current NST approaches. The NSTs are marketed as a targeted pesticide delivery system (Jeschke et al. 2011), however our findings demonstrate that NSTs may be a highly inefficient approach to applying active ingredients to plant tissues where insects will ingest them. In sum, less than 1.5% of the initial seed-applied AI was recovered in whole plant translocations. When looking at the root tissue alone, a maximum of 0.262% of the initial amount was translocated (Fig. 4), although it is unknown whether these levels are sufficient for pest management. For reference, a higher percentage of the initial ST amount applied to seeds has been reported as lost during planting due to abrasion (0.437%; Xue et al. 2015). The shoot region however translocated a maximum of 1.18%, a likely result of the high xylem mobility many of the neonicotinoids possess (Bonmatin et al. 2015). Similar translocation efficacy, under field conditions is expected for thiamethoxam, the second most widely-used NST in maize, which has a water solubility value approximately 10-fold higher than that of CLO (Jeschke and Nauen 2008, Huseth and Groves 2014). These results also contrast with those of Sur and Stork (2002) whose study reported 20% translocation of imidacloprid STs in maize. The authors mention that their translocation values may be inflated as the study was conducted in a greenhouse, reducing impacts of UV photolysis and weather conditions, and that the amount of soil in the plant boxes compared to the root mass exaggerated imidacloprid uptake. This is likely a key limitation of that study and may explain why our recovery from a field experiment was much lower; AI that would be lost to the water table in the field could remain in greenhouse enclosures and be available for uptake by plants throughout the season in this relatively closed system.

Determining the environmental fate of the remaining ca. 98% of active ingredient used in NST is an area primed for further research. The intrinsic characteristics of CLO, thiamethoxam and imidacloprid, and a growing body of literature reporting neonicotinoids in water lends support to the interpretation that the remainder of the ST is rapidly lost to the environment in ground and surface water (DeLorenzo et al. 2012, Starner and Goh 2012, Anderson et al. 2013, Sánchez-Bayo and Hyne 2013, Main et al. 2015). The high water solubilities of the compounds most commonly used in NST applications make it unlikely that they will reside near the target plant’s relatively limited rhizosphere long enough to be absorbed by the plant once they are not on the seed. This may explain why samples in 2015 generally had a higher % AI translocated (Fig. 5) as cumulative rainfall in 2015 was 2.66-fold less than over the same 20 DPP period in 2014. This may also explain why the concentrations reported in our study depart from those of Myresiotis et al. (2015). While plants were regularly irrigated in the Myresiotis et al. (2015) study, the authors mention they made sure the entire volume of water remained within the rhizosphere soil in an effort to avoid leaching. Using seed treated with 0.1 mg thiamethoxam kernel^{-1}, Myresiotis et al. (2015) found shoot concentrations of 0.62 and 0.13 ug thiamethoxam g^{-1} at 21 and 36 DPP respectively. Using the "Low" rate of 0.25 mg CLO kernel^{-1}, our study found in plant concentrations of 0.086 and 0.007 µg CLO g^{-1} at 20 and 34 DPP respectively in 2014. Despite the higher initial application rate of our seed, the Myresiotis et al. (2015) study had overall larger AI concentrations in shoot tissue which is likely due to differences in experimental design. AI that was lost to leaching in our field study would have been preserved in a potted plant study with constrained irrigation.

2.6 Conclusions

While the highest in-plant neonicotinoid concentrations reported from this research may provide some control of early season root and seed pests, the relatively brief window of high AI concentrations poorly coincides with the phenology of the key maize pest in the USA. This coupled with the sporadic occurrence of economic infestations (Royer et al. 2004) of secondary pests indicates that most US maize producers are unlikely to realize benefits from the NST approach. Furthermore, the widespread prophylactic application of NSTs (Douglas and Tooker 2015) and their high water solubility, coupled with the limited translocation efficiency reported in this study, provide a mechanism to explain increasing detections of NST compounds in non-target lands and waterways (DeLorenzo et al. 2012, Starner and Goh 2012, Anderson et al. 2013, Sánchez-Bayo and Hyne 2013, Main et al. 2015).

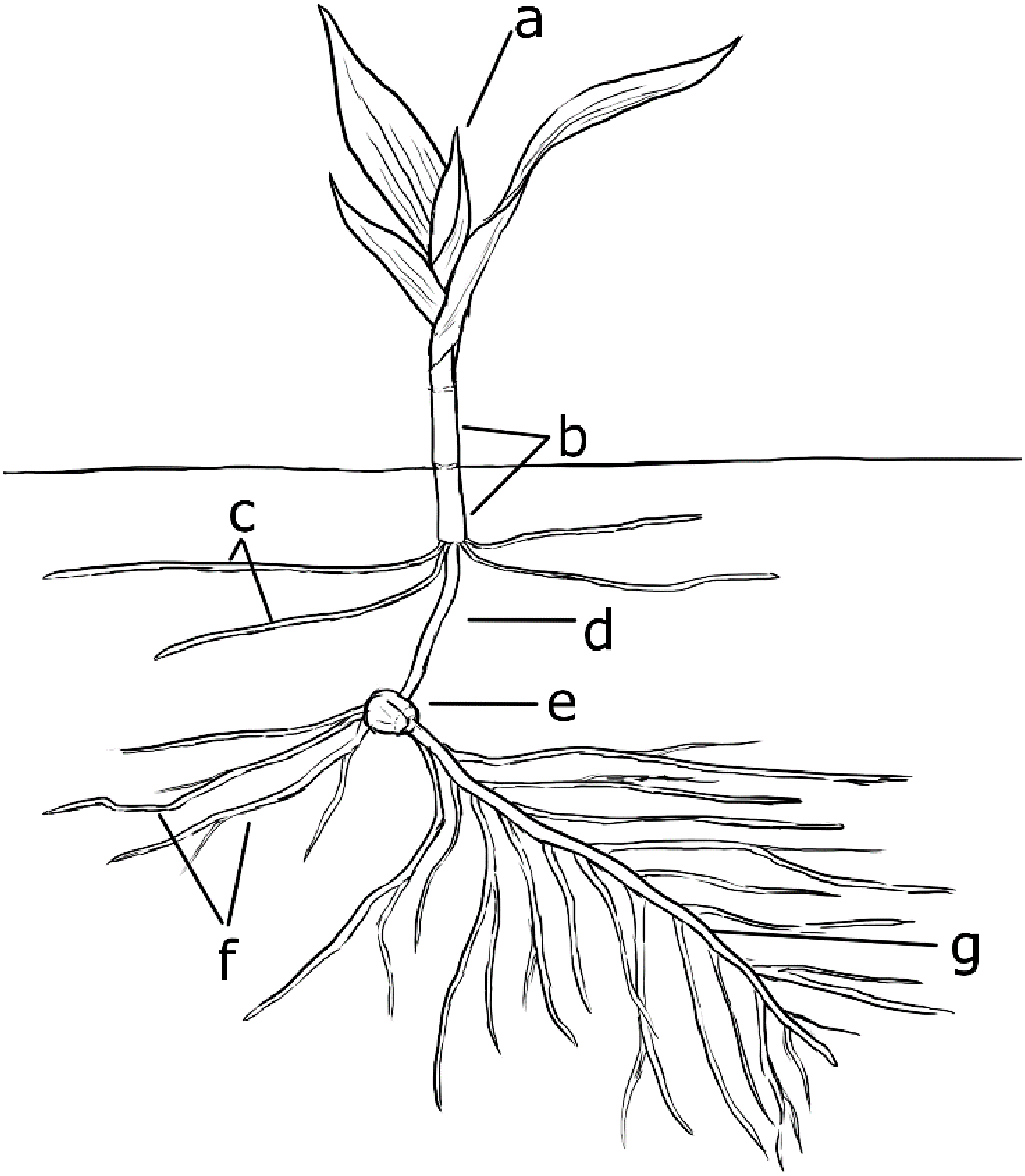


Figure 2. Diagram of a maize seedling at the soil surface interface, showing (a) Stem apex, (b) coleoptile, (c) nodal roots, (d) mesocotyl, (e) seed, (f) seminal roots, and (g) radicle roots. For homogenization purposes, the shoot region was classified as sections (a-d), the seed region as (e), and the root region as (f) and (g).

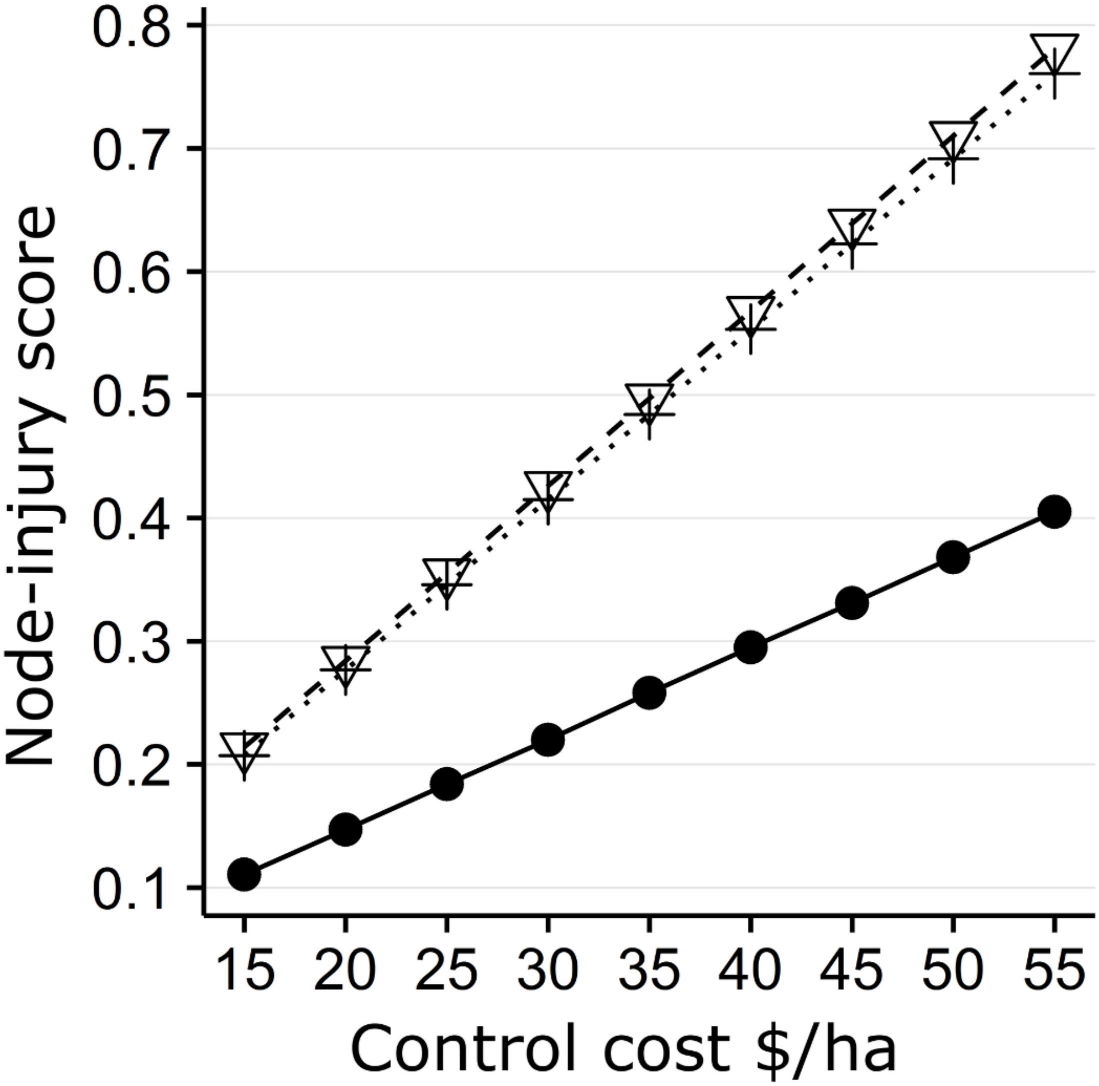


Figure 3. The minimum node-injury score estimated to cause economic damage calculated according to the Oleson et al. (2005) moderate environmental stress model. 2014 and 2015 are represented as an inverted triangle with dashed lines and crosses with dotted lines respectively. 2012 price data is included to show how recent (five year) high commodity values affect economic thresholds and is represented as a filled circle with solid lines.

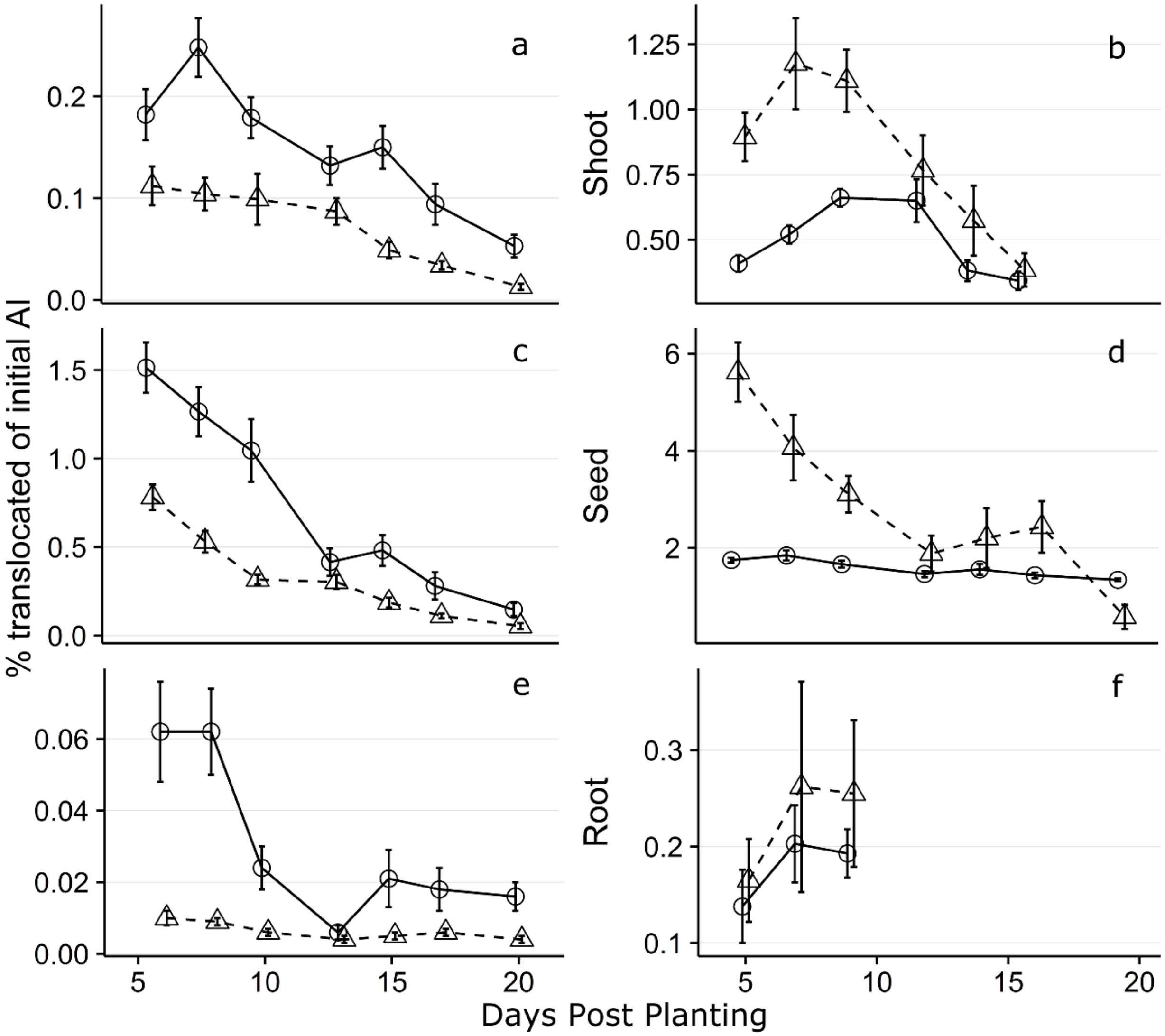
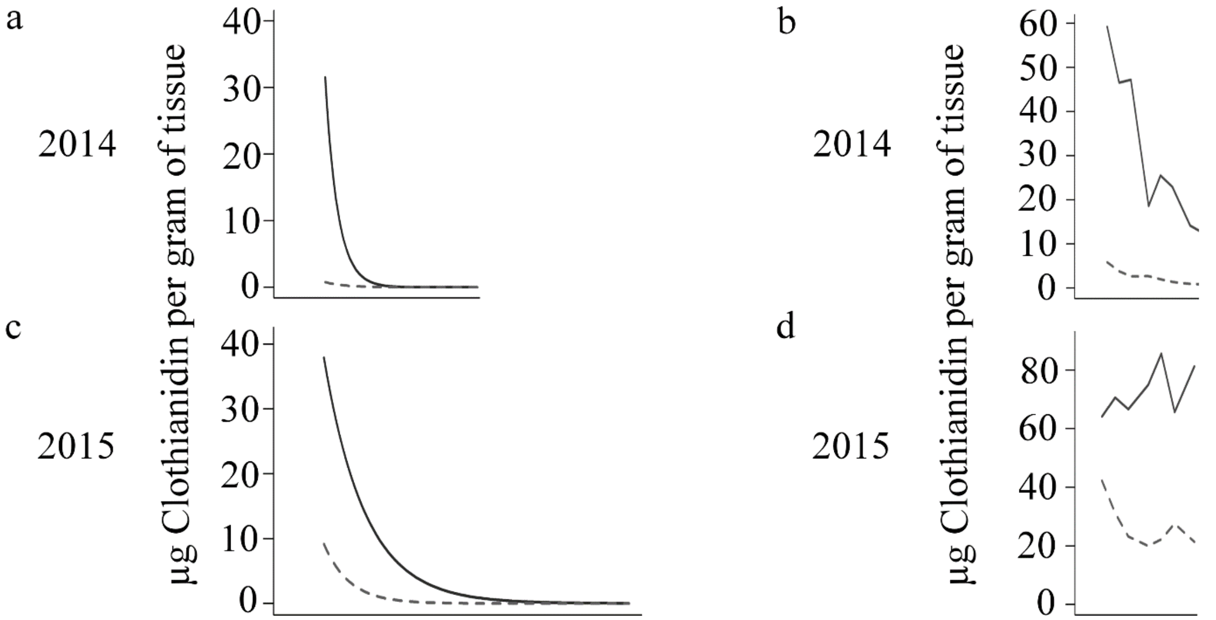


Figure 4. Mean percentage of initial clothianidin application translocated to root, seed, and shoot tissues. The “Low" and “High" treatment rates are represented by an open triangle with dashed lines and an open circle with a solid line, respectively. Only plants with >80% of root and >80% of shoot tissue were used in calculation of the % of initial AI translocated. The 2014 data are represented by graphs (a), (c), and (e), and 2015 data by graphs (b), (d), and (f). The first 20 d post planting (DPP) are shown. The 2015 plots received 2.66 fold less rainfall in the first 20 DPP leading to overall less “complete” root samples being collected.



Root Pest	May				June				July			
White Grub	▨	▨	▨	▨	▨	▨						
Corn Rootworm					▨	▨	▨	▨	▨	▨	▨	▨

Seed Pest	May			
Wireworm	▨	▨	▨	▨
Seedcorn Maggot			▨	▨

Figure 5. Values of µg clothianidin g^{-1} plant tissue fit to a first order decay equation with time as a predictor. The root region is represented by graphs a and c whereas the seed region by graphs b and d. Actual concentrations for the seed region are displayed given the poor fit of predicted values. Dashed and solid lines represent the 0.25 and 1.25 mg clothianidin $kernel^{-1}$ application rates. The pest activity period is displayed underneath the graphs with activity indicated by a filled in box.

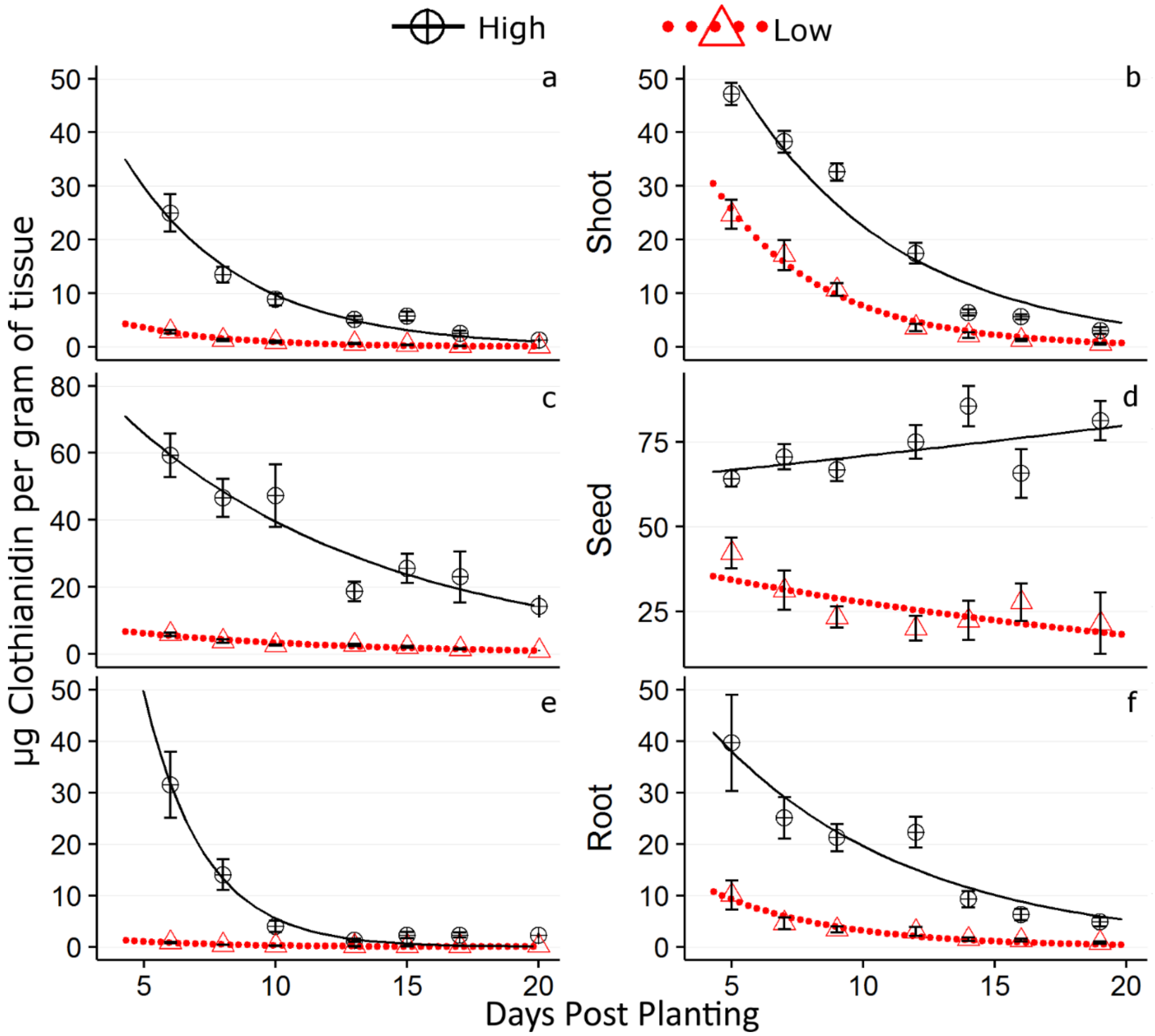


Figure 6. Values of µg clothianidin g^{-1} plant tissue with standard error bars. The 2014 data are represented by graphs (a), (c), and (e), and 2015 data by graphs (b), (d), and (f). The first 20 days post planting (DPP) are shown. Concentrations as predicted by the first order exponential decay equation are represented by a dotted red line and solid black line for the respective 0.25 and 1.25 mg clothianidin kernel^{-1} application rates.

CHAPTER 3: A META-ANALYSIS AND ECONOMIC EVALUATION OF NEONICOTINOID SEED TREATMENTS AND OTHER PROPHYLACTIC INSECTICIDES IN INDIANA MAIZE FROM 2000-2015 WITH IPM RECOMMENDATIONS

3.1 Introduction

Insecticide use is key component of crop protection in many commodities with pesticide use in general becoming the dominant approach to US pest control following WWII (Osteen 2003). This is in part due to a combination of low cost, effectiveness, and convenience or ease of application (MacIntyre 1987). However, insecticide use is not without some significant drawbacks, both ecological and economical. While the definition of IPM has evolved over the past 50+ years (Kogan 1998), an ideal modern IPM program would include monitoring and management of key pests (including weeds, insects, and fungal pathogens), use of multiple suppressive tactics in order to achieve economic levels of control, and the judicious use of pesticides where necessary as determined by economic thresholds (Ehler 2006). In reality, modern IPM in field crops such as maize and soybeans frequently falls short of this ideal, relying largely on prophylactic pesticide use to treat pests, with limited rotation of active ingredients to mitigate resistance (Ehler 2006). By 2011, preemptive insecticide use increased to 34-44% in soybeans and 79-100% of maize in the US while pest pressures have not increased over the same period (Douglas and Tooker 2015). While this approach may be partially dictated by current market efficiencies, it fails to address the root cause of pest problems (Ehler 2006). Despite this, the benefits of IPM have been well documented with a review covering 61 economic evaluations of IPM over a span of 20 years (1973-1993) in 8 commodity groups (Cotton, Soybeans, Corn, Vegetables and Flowers, Fruits, Peanuts, Tobacco, and Alfalfa), finding a 14.9% decrease in pesticide use, 2.8% decrease in production cost, an 11.4% increase in yield, and a 47.8% net return per acre (Norton and Mullen 1994).

In the Midwestern US, a variety of IPM strategies have been researched and developed (Levine and Oloumi-Sadeghi 1991) to deal with the primary pest of maize, the corn rootworm (CRW) (*Diabrotica virgifera*) (Gray et al. 2009). These include crop rotation (Levine and Oloumi-Sadeghi 1991), planting date alteration (Musick et al. 1980, Naranjo and Sawyer 1987), and varying tillage practices (Gray and Tollefson 1988a, 1988b). An increase in rotation resistant CRW populations (Krysan et al. 1984, Onstad et al. 1999, Levine et al. 2002), pesticide resistance (Ball and Weekman 1963, Meinke et al. 1998, Wright et al. 2000) and resistance to Bt corn hybrids (Gassmann et al. 2011) have combined to reduce the effectiveness and applicability of these IPM strategies. Foliar sprays have occasionally been used within an IPM framework with action thresholds at 1-1.57 beetles plant^{-1} in continuous corn, and 0.83 beetles plant^{-1} in first year corn (Pruess et al. 1974, Godfrey and Turpin 1983). These foliar sprays target adult CRW in an effort to reduce larval damage in the subsequent season and protect plant silks from adult feeding. However, the efficacy of this approach is heavily influenced by abiotic factors that dictate pest phenology (Naranjo and Sawyer 1989), including precipitation (Mayo 1984), and is likely to be more expensive than a single soil applied insecticide at planting if >2 sprays are needed (Bergman 1987). Furthermore, the sampling methods (Steffey et al. 1982, Foster et al. 1982) designed to accurately count adult beetles and inform action thresholds (Pruess et al. 1974, Godfrey and Turpin 1983) are labor intensive and poor predictors of economic damage and yield loss in the next year (Hein and Tollefson 1985, Foster et al. 1986). This disconnect has been attributed to a lack of basic research examining CRW population dynamics and insect plant interactions (Hein et al. 1988). For the latter part of the 20th century, prophylactic application of soil insecticides was found to be the most economically feasible approach to CRW management in continuous corn due to the difficulty in predicting larval populations (Foster et al. 1986).

With the diminished utility of traditional IPM practices, logistical hurdles such as those outlined above, along with the economic uncertainties associated with conducting a consistent IPM program, CRW management in commercial maize production has continued to move toward a prophylactic, insurance-based approach, frequently using Bt hybrids as the cornerstone (Gray 2011). Hybrids utilizing Bt technology have largely controlled CRW since their initial introduction in 2004-06 (Storer et al. 2006, Ma et al. 2009), although non-high dose toxins, variability of toxin expression in plant material and inadequate refuges have led to several cases of field-evolved Bt resistance in CRW (Gassmann et al. 2011, Gassmann et al. 2014). The move away from traditional IPM approaches in maize and soy crops is further manifested by the introduction and rapid adoption of neonicotinoid seed treatments (NST). As of 2011, over 80% of maize and 34-44% of soy planted in the USA is treated with either clothianidin (CLO) or thiamethoxam (THX) as a seed treatment at application rates of 0.25-1.25 mg kernel^{-1} prior to sale to the grower (Krupke et al., 2012; Douglas and Tooker 2015). The sharp increase in NST use has not been in response to any increase in pest damage or threat (Douglas and Tooker 2015). This runs contrary to a key principle of IPM (Pedigo and Rice 2009), that prioritizes the judicious use of pesticides as a means forestalling resistance and limiting the likelihood of negative effects on both human and environmental health.

NSTs have been marketed as a highly versatile and effective insecticide group with relatively low risk to non-target organisms in comparison with older insecticide classes (Jeschke and Nauen 2008). While the 1.25 mg kernel^{-1} rate of clothianidin is labeled for control of the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte), NSTs are also labeled for other early season secondary pests attacking seeds and the developing root tissue (Jeschke et al. 2011) including wireworms (Riley and Keaster 1979), seedcorn maggots (Higley and Pedigo 1984), and white grubs (Jordan et al. 2012). Both seedcorn maggots and wireworms preferentially attack the seed region of young plants early in the season (Riley and Keaster 1979, Higley and Pedigo 1984) whereas white grubs and CRW attack the roots (Metcalf and Metcalf 1993) causing plant lodging, reduced water uptake and increasing potential for yield loss (Levine and Oloumi-Sadeghi 1991). The vast majority of these secondary pests are not relevant to most producers as economic infestations are erratic and difficult to predict (Royer et al. 2004), with the exception of seedcorn maggot infestations, which can be reliably anticipated based on the incorporation of a green cover crop (Hammond 1990). Recent research (Alford and Krupke 2017) indicates that the period when neonicotinoid residues are present within plant tissues offers a good fit with the phenology of some secondary pests, but does not align well with the phenology of the Western corn rootworm, the primary insect pest of North American maize production.

While multiple reviews have addressed the non-target effects of neonicotinoids upon a range of organisms, including pollinators, migratory waterfowl and aquatic invertebrates (Goulson 2013, Nuyttens et al. 2013, Godfray et al. 2014, Morrissey et al. 2015, Pisa et al. 2015), only one review has compared the crop protection potential of neonicotinoids against that of previous insecticide classes; this is surprising given the rapid adoption and widespread use of neonicotinoids in maize. Tinsley et al. (2015) utilized maize insecticide efficacy trial data from 2003-14 from Illinois and Nebraska trials to describe the damage reduction attributable to various management strategies. Treatments included NSTs at the CRW rate (1.25 mg CLO kernel^{-1}), soil insecticides, single-toxin Bt maize (± soil insecticide), and dual-toxin Bt maize (± soil insecticide). Data were analyzed by pairing the node injury for each insecticidal treatment with that of the untreated control for each study location. A regression of that panel data was then used to create efficacy equations. Soil insecticides, including both granular and liquid formulations, were grouped together regardless of application rate or active ingredient and included organophosphates, phenylpyrazoles, and pyrethroids. Bt hybrids were also treated with a low, “non-CRW”, rate (0.25-0.50 mg ai kernel^{-1}) of NST. The dual-toxin Bt maize + a soil insecticide approach led to the greatest significant reduction in larval CRW damage (97%), while the NSTs led to smallest damage reduction (48%). Soil insecticides performed about as well as single toxin Bt alone with respective reductions of 72% and 78% suggesting single toxin Bt without a soil insecticide could be rotated with soil insecticides alone to extend the utility of both approaches. Another conclusion of Tinsley et al. (2015) was that CRW rates of NSTs (1.25 mg ai kernel^{-1}) are unlikely to provide adequate protection at higher CRW densities and their associated high damage potential, compared with other available options.

The objective of my analysis is to compare NSTs to previously and currently utilized prophylactic soil insecticides in order to assess their overall effectiveness across multiple agronomic parameters in maize. Unlike Tinsley et al. (2015), soil insecticides were grouped by active ingredient (AI) and application rate prior to analysis in order to estimate the value of products available in the maize insecticide market. My data source is comprised of 15 years (2000-15) of insecticide efficacy trials in maize conducted by the Purdue entomology field crops lab. These trials were conducted annually to provide growers an unbiased source of efficacy for maize insecticides currently available to them. As the sample size, variance, and mean are known for each treatment, these data are readily analyzed within a meta-analytical framework, allowing us to compare the overall mean effect of each AI and rate. Finally, I calculated the probability of a grower financially recouping seed and insecticide costs for each insecticide/rate by using the overall insecticide associated yield increase, and a range of current insecticide and maize sell prices. This analysis is spurred on by both the unprecedented use rates of NSTs in maize and the growing reports of Bt resistance among CRW populations at various locations throughout the corn belt. The results will allow us to explore and assess the most appropriate insecticide options for producers in regions with varying levels of CRW pressure and Bt resistance.

3.2 Materials and methods

3.2.1 Source data

Datasets comprising insecticide efficacy studies conducted yearly from 2000-2015 by the Purdue entomology field crops lab were used. These studies were carried out across 5 agricultural field stations in the State of Indiana, representing a cross-section of the regions where the majority of maize production in the state occurs: Pinney Purdue Agricultural Center (PPAC) at 41˚26'35.22"N 86˚55'48.34"W, the Northeast Purdue Agricultural Center (NEPAC) at 41˚6'15.43"N 85˚23'55.67"W, Davis Purdue Agricultural Center (DPAC) at 40˚15'12.07"N 85˚8'52.92"W, Throckmorton Purdue Agricultural Center (TPAC) at 40˚17'48.56"N 86˚54'11.26"W, and Southeast Purdue Agricultural Center (SEPAC) at 39˚2'12.49"N 85˚31'42.58"W. Trial plots were conducted in both large and small maize plot arrangements in a randomized complete block design (RCBD) with four blocks in all years. Large plot studies were conducted in years 2000-07 with a given treatment consisting of 3 adjacent rows, 70.4-183 m in length depending on the year and location. Small plot trials were conducted in all years with a treatment consisting of a single row, 30.5 m in length. Both plot types were included in the meta-analysis. Of the 69 efficacy trials used in this project, soybean was used as the previous crop in only 8 efficacy trials. The previous seasons crop in all other trials was either maize (n=21) or a late-planted maize trap crop (n=40) in order to maximize the probability of CRW egg deposition and larval feeding pressure for the following season's efficacy trial. Experimental plots were planted with a variety of tillage methods (spring chisel plow, disk, field cultivator, etc) representative of recommended practices, please see Appendix B for additional details. Herbicides were applied as needed and following local agronomic practices.

3.2.2 Selection criteria

The following *a priori* criteria were selected for inclusion of a treatment in the meta-analysis. First, a treatment must have been used for a minimum of 3 growing seasons irrespective of location. Second, a treatment must have been used at a minimum of 2 locations. Third, only treatments with greater than 10 separate treatment means were included. These considerations were included to limit the effect of extreme growing conditions (drought, flood, high growing temperature, etc.) across space and time and their effects upon variability. All insecticides were

Table 6. Compounds included in meta-analyses. All GUS values reported from Pesticide Properties Database (2017). Estimates for clothianidin costs were estimated using data from Studebaker (2007) and North et al. (2017).

Compound	Rate (mg AI m^{-1})	Treat. abb.	IRAC group	GUS risk[1]	Years used	Cost \$ ha^{-1} X±SD, (n)
Bifenthrin	6.98	BIFEN Low	3A	Low	00-07	35.32±5.60 (9)
Bifenthrin	8.56	BIFEN High	3A	Low	01, 03-05	42.78±6.55 (9)
Chlorethoxyfos	13.95	CHLETH Low	1B	Low	00-03	N/A
Chlorethoxyfos	17.21	CHLETH High	1B	Low	02-07	N/A
Chlorpyrifos	111.61	CHLPYR	1B	Low	00-07, 10, 11, 13-15	43.47±10.19 (7)
Cry3Bb1	N/A	CRY3Bb1	11A	N/A	03-04	N/A
Fipronil	11.16-12.09	FIP	2B	Med	00-06	N/A
Clothianidin	6.52-7.77	1.25 CLO	4A	High	01-11, 13-15	17 to 52
Imidacloprid	6.99-8.33	IMID	4A	High	00-02	N/A
Tebupirimphos/ Cyfluthrin	13.02-13.67	TEBU/CY	1B/3A	Med/ Low	00-07, 10, 11, 13-15	62.87±6.63 (15)
Tefluthrin	11.161	TEFLU	3A	Low	00-07, 10, 11, 13-15	63.08±8.92 (11)
Terbufos	111.61	TERB	3A	Low	00-04, 07, 11	65.55±7.67 (7)

converted to AI m^{-1} to standardize and group granular and liquid insecticides across trade names and delivery methods whereas insecticidal seed treatments were grouped by active ingredient per kernel, as AI m^{-1} would vary with plant population (i.e. seeds m^{-1} of planted row) (Table 6). Small plot studies would often provide multiple treatment means of a given compound AI m^{-1} as multiple delivery methods (liquid versus granular), delivery locations (parallel to or in line with the seed), insecticide brands ("name" brand versus generic), and/or hybrids may have been tested within a given study. Large plot studies only provided one treatment mean per compound AI m^{-1}.

3.2.3 Meta-analytical model

Analyses of yield, stand count, and root damage were conducted on all studies that fit the above selection criteria. While yield is the most critical ultimate measure of pesticide effectiveness, it does not allow for assessment of when or how pest damage occurs. This omission can lead to a higher probability of incorrectly attributing a yield increase to a pesticide choice when it is really the result of compensatory growth masking pest damage (Kahler et al. 1985, Lemcoff and Loomis 1994), sub-economic levels of insect feeding or other factors. To increase resolution, stand counts may be performed early in the growing season to indirectly assess the abundance of early season pests and/or phytotoxicity by comparing the number of kernels planted with the percentage of plants found in a subsequent survey. While two different CRW scales (0-3 point (Oleson et al. 2005) and the Iowa 1-6 scale (Hills and Peters 1971)) were used to quantify CRW damage to maize plants, no detailed methods have been developed for secondary pests, perhaps because they are not commonly found at economically damaging levels in most fields (Royer et al. 2004). Consequently, only CRW damage was assessed. Analyses were conducted on both the 0-3 scale in years 2002-15 and the 1-6 scale from 2000-04; the 0-3 scale was fully implemented as the standard beginning in 2005.

All meta-analyses and effect sizes were calculated with the *metafor* package version 1.9-9 (Viechtbauer 2010) in R 3.4.0 (R Core Team 2017). Effect sizes (Hedges' *g*) were calculated with the *escalc* function using the standardized mean difference estimator. In addition to correcting for small sample sizes, this approach divides the difference between the treatment mean and comparator mean, by the pooled variance in order to produce an effect size (Hedges 1981). Two different comparators were used, an untreated control (UTC) and a Poncho 250 rate, in which the rate of 0.25 mg $kernel^{-1}$ of CLO was applied (0.25 CLO). While 0.25 CLO contains a nominal level of insecticide and is thus not a true “control”, the inclusion of this comparison set is justified as the 0.25 CLO rate is the lowest rate of treated seed conventional growers have access to; untreated seed is largely absent as an option for US maize producers (Krupke et al. 2012, Douglas and Tooker 2015). Furthermore as an UTC was unavailable for comparison in some years, inclusion of a 0.25 CLO comparator allowed us to utilize the data from these years. All other years (00-06, 10, 15) used an UTC for effect size calculation, however, whenever 0.25 CLO was used in years alongside an UTC (01, 02, and 15), that data was also used in the 0.25 CLO comparisons. Most of the 2008 data could not be used as most site/years planted seed treated with a 0.25 CLO rate in addition to any further applied soil insecticides. This management decision made it impossible to separate the joint 0.25 CLO/insecticide effect into its respective constituents. Similarly, 2012 data could not be used as no control (0.25 CLO or UTC) was used that year.

3.2.4 Random-effects model

The *rma.mv* function in *metafor* was used to calculate the results of each meta-analysis with a restricted maximum likelihood estimator. A random-effects model was first used to assess the overall effect of insecticides at CRW rates and used “site-year” as a random factor. A site-year was defined as all comparisons that took place within the same trial, year, and location. For example, even if a given trial had two different hybrids with respective controls (as in 2003 small plots, where hybrids RX708 and NK N72-V7 were planted together), they would still be considered the same site-year as they shared all site attributes except hybrid, although a hybrid-specific effect size was still calculated. If an agricultural center had both large and small plots planted within the same year, they would be considered separate site-years.

Concern of publication bias, otherwise known as the “file drawer problem” in which non-significant studies are not published (Rosenthal 1979), is not a key concern, as none of these analyses were published previously in peer-reviewed journals. Despite this, and to remain conservative in our analysis, the fail-safe number for each significant analysis was calculated according to Rosenthal’s method (1979) at an α of 0.05 and represents the number of non-significant studies that would need to be included in the meta-analysis to change the results from significance to non-significance. Fail-safe numbers are considered robust if $>5n+10$ with n representing the number of studies within the meta-analysis.

3.2.5 Mixed-effects model

Following the random effects models, each plant health metric was analyzed with a mixed model using "AI m^{-1}" as a fixed categorical factor and "site-year" as the random factor. A list of tested compounds and abbreviations can be found in Table 6. Differences between each tested insecticide were assessed with an omnibus test of between-group heterogeneity (Q_b) and compared against a χ^2 distribution with differences considered significant at $P<0.05$. The mean effect was plotted along with confidence intervals and treatment significant differences were determined from overlap of confidence intervals with both the comparator line and other mean AI m^{-1} effect sizes.

3.2.6 Economic analysis

To facilitate economic analyses across all site-years, the proportional yield was calculated for each treatment mean within a given site-year. This was done by dividing each treatment's mean yield, by the treatment with the largest treatment mean yield, for the respective hybrid at that given site-year. In this manner, yields represent a proportion of the maximum treatment yield in that site-year. Next, a treatment-associated yield benefit was calculated by taking the difference between a treatment's mean proportional yield, and the mean proportional yield of the corresponding control. All yield benefit data for a given compound were then analyzed with a t-test to calculate the mean treatment effect (TE) and its standard deviation (TE_{SD}) as in Esker and Conley (2012) and Krupke et al. (2017). Both variables (TE and TE_{SD}) were used to calculate the mean net expected return (μ) and its standard deviation (σ) in \$/ha as follows:

$$\mu = MP \ x \ Y \ x \ TE - IC$$

and

$$\sigma = MP \ x \ Y \ x \ TE_{SD}$$

where Y represents the average Indiana yield from 2010-15 (\$9.59 mt^{-1}; USDA-NASS 2017b), MP as the average Indiana sale price from 2010-15 (\$203.93 mt^{-1}; USDA-NASS 2017c). A five year average for both Y and MP was used in order to account for annual variability in prices. Insecticide cost was represented by IC and was estimated with telephone surveys conducted in January 2017 with various vendors listed in the Indiana State Chemist's Restricted Use Pesticide dealer database. Vendors were selected based on proximity to each of the five agricultural field stations and must have been adjacent to or within the same county as the agricultural field station. Data from at least

2 vendors per research station were used. Current (winter 2016) insecticide prices were reported as none of the surveyed vendors were able to provide historical price data.

Both μ and σ were used to parameterize a probability density function of the form:

$$f(x;\mu,\sigma^2) = (1/\sqrt{2\pi}(\sigma*\sigma))\ e^{-0.5(x-\mu/(\sigma*\sigma))}$$

Finally the cost relative yield (*CRY*) was calculated as:

$$CRY = IC/(MP\ x\ Y)$$

representing the minimum yield increase percentage needed to recover treatment costs and serves as a point within the probability distribution to calculate the one-tail breaking even probability.

The average price of *MP* and *IC* ± 1 standard deviation was used to provide a price range upon which economic feasibility could be calculated (Table 6) resulting in the *CRY* being calculated under 9 different economic conditions (3 different *MP* x 3 different *IP*) for each insecticide. The 1.25 CLO rate could not be estimated as NST are applied prior to sale to the grower and the cost of this service is not disclosed in the marketing of maize seed in North America. Estimated costs range from \$52.36 ha^{-1} (North et al. 2017), ~\$39.5-47 ha^{-1} (Shields 2003), \$37.5 ha^{-1} (Seagraves and Lundgren 2012), ~\$34.6-42 ha^{-1} (Bessin 2003), and ~\$17 ha^{-1} (Studebaker 2007) so the break-even probability was calculated under prices similar to this range.

3.3 Results

3.3.1 Random-effects model

Use of a CRW rate insecticide, regardless of AI, led to significant decreases in node damage and significant increases in stand and yield as evidenced by the overall effect on each plant health metric with an UTC comparator (Table 7). Of the two metrics assessed with a 0.25 CLO comparator, only node damage was significantly reduced, with no significant change in stand count from the 0.25 CLO comparator (Table 7). The mean effect of all tested CRW insecticides resulted in significant decreases in root damage (Oleson: Hedge's $g = -1.86$ $P < 0.0001$; Iowa: Hedge's $g = -1.84$ $P < 0.0001$), and significant increases in yield (Hedge's $g = -0.82$ $P < 0.0001$ when an UTC was used. Only root damage was significantly reduced with a 0.25 CLO comparator (Hedge's $g = -0.97$ $P = 0.001$). All significant models had robust fail-safe numbers >5000, indicating a large number of non-significant studies would need to be included to change significant results to non-significance (Table 7).

Table 7. Mean effect size (*g*) of insecticide related changes in various crop health metrics using two different comparators (UTC and 0.25 CLO). All models assessed the effect of insecticides at CRW rates and used the No. of site-years as a random factor. The fail-safe number (FSN) was calculated using the Rosenthal method (1979).

Comparator	Metric	*g*	CI	*Z*	*P*	No. site years	FSN
UTC	Stand	0.16	0.15	2.15	0.03	55	8778
UTC	Oleson	-1.86	0.43	-8.58	<0.0001	40	420035
UTC	Iowa	-1.84	0.33	-11	<0.0001	35	419124
UTC	Yield	0.82	0.23	6.97	<0.0001	31	22137
0.25 CLO	Stand	-0.004	0.34	0.02	0.98	21	0
0.25 CLO	Oleson	-0.97	0.58	-3.3	0.001	18	5211

3.3.2 Mixed effects model

Significant heterogeneity was observed between effect size means in models analyzing root damage (Oleson: $Q_b = 18.333$ $P = 0.049$; Iowa: $Q_b = 44.317$ $P < 0.0001$), stand ($Q_b = 48.265$ $P < 0.0001$), but not in yield ($Q_b = 5.502$ $P = 0.703$) with the use of a UTC comparator. In the stand model, the 1.25 CLO effect size mean significantly increased stand counts in comparison to most other effect size means (Fig. 7b) as determined by CI overlap. As the effect size CI of each AI/m overlaps in the Oleson model, it is impossible to determine where between group differences lie (Fig. 8a). In contrast, both the TEBY/CY and Cry3Bb1 treatments significantly reduced root damage on the Iowa scale in comparison to the FIP mean effect size (Fig. 8b). When a P250 comparator was used to describe stand changes and root damage, significant heterogeneity was only observed in the stand model (Stand: $Q_b = 14.789$ $P = 0.011$; Oleson: $Q_b = 5.690$ $P = 0.224$). It is likely the 1.25 CLO treatment significantly increases stand counts in comparison to at least one other AI m^{-1} effect size mean, but again, as the CI of each AI m^{-1} effect size overlaps, it is impossible to determine which comparisons are significant.

When compared to the UTC effect line (Hedge's $g = 0$), all tested CRW insecticides significantly increased yield (Fig. 7a.), and decreased node damage on both the Oleson (Fig. 8a.) and Iowa (Fig. 8b.) scale as evidenced by the lack of overlap of CI with UTC effect line. In the UTC stand model (Fig. 7b.), all insecticides with the exception of IMI and 1.25 CLO did not significantly increase stand in comparison to an UTC. When insecticides were tested with a P250 comparator, all tested insecticides significantly decreased root damage (Fig. 9a), but did not significantly alter stand counts (Fig. 9b).

3.3.3. Economic analysis

Six compounds could be assessed with the economic analysis (Table 8). Both FIP and the CHLETH treatments (Table 6) were not included despite their inclusion in the meta-analysis (Fig. 6a) as these compounds have been off market for several years and no price estimates were available. A high probability (>80%) of breaking even was associated with all tested compounds under varying market conditions (Table 8). The *CRY* for all treatments was <5% in the vast majority of cases indicating an insecticide would only have to increase the yield by a marginal amount in order to recover the seed and insecticide costs (Table 9). Likewise, the expected economic return for each treatment associated mean is presented in Table 10.

3.4 Discussion

This analysis reveals that while the NST treatment was the only set of compounds to result in higher stand counts (Fig. 6b), this advantage was not borne out by other comparison parameters, including yield. The NSTs 1.25 CLO and IMI were the only compounds tested that led to significantly higher stand counts with an UTC comparator (Fig. 6b), but not with a 0.25 CLO comparator (Fig. 9b), although the presence of fungicide seed treatments in these treatment is an important confounding factor. All other compounds failed to increase stand in comparison to their respective comparator (UTC: Fig. 6b; 0.25 CLO: Fig. 9b). Given the overlap between 1.25 CLO CI with a 0.25 CLO comparator line (Fig. 9b), it is also a possibility that the stand protection NSTs provide is not rate dependent, at least between these two rates. There were too few studies that included a 0.25 CLO rate in the UTC meta-analyses so I cannot determine if the 0.25 CLO rate significantly increases stand counts in comparison with the 1.25 CLO rate. Interestingly, while this finding reaffirms young plant protection can be provided by NSTs (Elbert et al. 2008), the 1.25 CLO stand increase did not lead to higher yields than compounds that did not increase stand (Fig. 6a). This observation is possibly the result of compensatory growth in treatments with lower stand counts, which has been well documented in maize (Kahler et al. 1985, Lemcoff and Loomis 1994), or sub-economic levels of feeding. Finally, it is important to note that the stand increase may be at least partially attributable to the suite of fungicides applied to seeds with NSTs. All other

Table 8. Breaking even probability of different prophylactic insecticides under varied maize sale prices (2010-15 Indiana sale price $\bar{X}$ ± 1 SD) and insecticide costs (Vendor $\bar{X}$ ± 1 SD). Estimates of the 1.25 CLO treatment range from ~\$17 ha^{-1} (Studebaker 2007) to ~\$52.36 ha^{-1} (North et al. 2017) so a range of prices was tested.

Maize Sell Price		**Insecticide cost (\$ ha^{-1})**				**Insecticide cost (\$ ha^{-1})**		
(\$ mt^{-1})	**Compound**	**29.72**	**35.31**	**40.92**	**Compound**	**33.28**	**43.47**	**53.66**
149.21	BIFEN Low	0.993	0.989	0.982	CHLPYR	0.997	0.992	0.981
203.93	BIFEN Low	0.996	0.995	0.993	CHLPYR	0.999	0.998	0.995
258.26	BIFEN Low	0.998	0.997	0.996	CHLPYR	0.999	0.999	0.998
	Compound	**56.23**	**62.86**	**69.50**	**Compound[1]**	**20.00**	**40.00**	**60.00**
149.21	TEBU/CY	0.954	0.926	0.885	1.25 CLO	0.995	0.983	0.946
203.93	TEBU/CY	0.988	0.981	0.980	1.25 CLO	0.997	0.991	0.978
258.26	TEBU/CY	0.995	0.992	0.989	1.25 CLO	0.998	0.994	0.988
	Compound	**57.88**	**65.55**	**73.22**	**Compound**	**54.16**	**63.08**	**72.00**
149.21	TERB	0.912	0.871	0.818	TEFLU	0.989	0.974	0.944
203.93	TERB	0.965	0.950	0.931	TEFLU	0.999	0.995	0.990
258.26	TERB	0.981	0.974	0.965	TEFLU	0.999	0.999	0.997

insecticide treatments did not receive a fungicide treatment in the vast majority of site-years used in this study, whereas the NSTs likely did. I cannot definitively conclude that a seed applied fungicide was included with the NSTs used in the project as the bag tags, which detail the active ingredients within the seed treatment, had been discarded years before this project began. Despite this, NSTs are rarely sold without included seed treated fungicides and therefore I believe this is a relatively safe assumption. This is a key limitation of the study and means that it is not possible to separate out the relative contributions of NST and fungicide to stand increases I report here; future studies focused on untangling the relative contributions of NST and seed-applied fungicides would be useful.

Another finding worth noting is the overall similarity of results between the Oleson and Iowa scales with an UTC (Fig. 8). While significant differences were recorded in the Oleson model, the overlap of CI between tested compounds made determining treatment differences impossible. This was not the case for the Iowa scale where the Cry3Bb1 and TEBU/CY treatments decreased damage more than the BIFEN High treatment (Fig. 8b).

Table 9. The cost relative yield of different prophylactic insecticides under varied maize sale prices (2010-15 Indiana sale price $\bar{X}$ ± 1 SD) and insecticide costs (Vendor $\bar{X}$ ± 1 SD). This represents the minimum yield increase percentage needed to recover treatment costs.). Estimates of the 1.25 CLO treatment range from ~\$17 ha^{-1} (Studebaker 2007) to ~\$52.36 ha^{-1} (North et al. 2017) so a range of prices was tested.

Maize Sell Price		**Insecticide cost (\$ ha^{-1})**				**Insecticide cost (\$ ha^{-1})**		
(\$ mt^{-1})	**Compound**	**29.72**	**35.31**	**40.92**	**Compound**	**33.28**	**43.47**	**53.66**
149.21	BIFEN Low	2.08	2.47	2.86	CHLPYR	2.33	3.04	3.75
203.93	BIFEN Low	1.52	1.81	2.09	CHLPYR	1.70	2.22	2.75
258.26	BIFEN Low	1.20	1.42	1.65	CHLPYR	1.34	1.75	2.16
	Compound	**56.23**	**62.86**	**69.50**	**Compound**[1]	**20.00**	**40.00**	**60.00**
149.21	TEBU/CY	3.93	4.40	4.86	1.25 CLO	1.40	2.80	4.20
203.93	TEBU/CY	2.88	3.22	3.56	1.25 CLO	1.02	2.05	3.07
258.26	TEBU/CY	2.27	2.54	2.80	1.25 CLO	0.81	1.61	2.42
	Compound	**57.88**	**65.55**	**73.22**	**Compound**	**54.16**	**63.08**	**72.00**
149.21	TERB	4.05	4.58	5.12	TEFLU	3.79	4.41	5.04
203.93	TERB	2.96	3.35	3.75	TEFLU	2.77	3.23	3.68
258.26	TERB	2.33	2.64	2.95	TEFLU	2.18	2.54	2.90

This minor difference may be due in part to a difference in datasets analyzed (Iowa: 2000-04; Oleson: 2002-07, 10, and 15). However this explanation is unlikely as high CRW pressure (defined as an UTC root rating >0.5 and >3 on Oleson and Iowa scales respectively) was reported in a similar proportion of site-years in both analyses (Iowa: 62.9%; Oleson: 76.7%). Additionally, 20 of the 35 and 43 site-years were shared between the Iowa and Oleson scale analyses, respectively, limiting the impact of non-shared years. A more likely scenario is how both scales grade damage. The Iowa scale is qualitative and non-linear whereas the Oleson scale is quantitative and linear (Oleson et al. 2005). Therefore, a root with an injury score of 3 exhibits double the injury of a root with a score of 1.5 on the Oleson scale, unlike the Iowa scale. In addition to its nonlinearity, the

Iowa scale poorly describes injury equivalent to < 1 node. As a result, the effect size of a given treatment is artificially inflated when the comparator has > 1 node worth of damage and a treatment has <1 node of damage. A more complete description of how the Oleson and Iowa scale differ in how injury is scored can be found in Oleson et al. (2005).

Table 10. The mean expected economic return per hectare (\$ ha^{-1}) as a consequence of using a prophylactic insecticide in comparison to an UTC. Calculations used varied maize sale prices (2010-15 Indiana sale price $\bar{X} \pm 1$ SD) and insecticide costs (Vendor $\bar{X} \pm 1$ SD). Estimates of the 1.25 CLO treatment range from ~\$17 ha^{-1} (Studebaker 2007) to ~\$52.36 ha^{-1} (North et al. 2017) so a range of prices was tested

Maize Sell Price		**Insecticide cost (\$ ha^{-1})**				**Insecticide cost (\$ ha^{-1})**		
(\$ mt^{-1})	**Compound**	**29.72**	**35.31**	**40.92**	**Compound**	**33.28**	**43.47**	**53.66**
149.21	BIFEN Low	80.3	74.7	69.1	CHLPYR	79.6	69.5	59.3
203.93	BIFEN Low	120.7	115.1	109.5	CHLPYR	121.1	110.9	100.7
258.26	BIFEN Low	161.1	155.5	149.9	CHLPYR	162.5	152.3	142.1
	Compound	**56.23**	**62.86**	**69.50**	**Compound[1]**	**20.00**	**40.00**	**60.00**
149.21	TEBU/CY	45.8	39.2	32.6	1.25 CLO	104.4	84.4	64.4
203.93	TEBU/CY	83.4	76.7	70.0	1.25 CLO	150.0	130.0	110.0
258.26	TEBU/CY	120.7	114.1	107.5	1.25 CLO	195.7	175.6	155.6
	Compound	**57.88**	**65.55**	**73.22**	**Compound**	**54.16**	**63.08**	**72.00**
149.21	TERB	46.5	38.8	31.2	TEFLU	58.8	49.9	40.9
203.93	TERB	89.8	77.1	44.8	TEFLU	100.2	91.3	82.4
258.26	TERB	123.1	115.4	107.7	TEFLU	141.6	132.7	123.8

Of course, it is possible that pest pressure differences between these year ranges can account for part of this result. One hypothesis is that pests were more common prior to widespread NST and Bt use and the comparatively lower pressure in later sampling years is the result of area-wide suppression, as shown in the European corn borer response to widespread Bt adoption (Hutchison et al. 2010). For example, Bt maize hybrids targeting CRW were first introduced during the growing season of 2004, and their rapid and widespread adoption may have led to regional declines in CRW pressure. Similarly, it has been suggested that widespread NST adoption in soybeans has led to area-wide suppression of soybean aphid (Bahlai et al. 2015). Although no data exist to support or refute this hypothesis in CRW or other, secondary pests of maize, it is plausible, given the rapid and nearly complete adoption of NST in maize, soybeans and other annual crops over the last 10-15 years.

Our economic results differ slightly from a recent analysis on the economic benefits of NST in the mid-south from 2001-2014 which found NST increased yield by an average of 700 kg ha^{-1}, corresponding to an 8.2% increase in yield and an economic return of \$56 ha^{-1} when compared to a fungicide only treatment (North et al. 2017). In contrast, our NST associated yield increase was 8.7%, with a corresponding economic return of \$64.4-195.7 ha^{-1} under each of our *IC* and *MP* combinations (Table 10) when compared to an UTC. Assuming a NST cost of \$52.36 ha^{-1} for the CRW rate (North et al. 2017), our economic return changes to \$72, \$117.7, and \$163.3 ha^{-1} for our respective price points of \$149.21, \$203.93, and \$258.26 mt^{-1} (calculations not shown in table). Part of why our economic return differs from North et al. (2017), despite a similar increase in yield, is how both studies calculated the yield sale price. In North et al. (2017), the yield of each treatment (NST, fungicide) was multiplied by the average sale price of the corresponding year and state for each efficacy trial, with the cost of the NST being subtracted from the gross economic return. As maize prices fluctuated widely over the 2001-2014 time period in the mid south (min: \$80 mt^{-1} max: \$290 mt^{-1}), years in which large economic returns were reported are partially suppressed by years in which smaller economic returns were reported. In contrast, our study took the average maize sale price Indiana growers received from 2010-2015 which included historically high maize sale prices. This artificially increased our mean *MP* (\$203.93 mt^{-1}) but I accounted for this by testing a range of *MP*.

Our tests were conducted using commercially available products and rates representative of what growers in the state of Indiana may use to manage key pests. Across all studies, the 1.25 CLO AI m^{-1} ranged from 6.52-7.77 mg m^{-1} corresponding to a planting rate of 38910-46,354 KPH meaning only the low BIFEN treatment possessed a lower AI m^{-1} (Table 6). While this finding suggests that ST use decreases the high dose rates associated with in-furrow application or sprays (Elbert et al. 2008), the rapid and thorough adoption of NSTs has actually increased the percentage of maize hectares treated with insecticide (>75% in 2011) (Douglas and Tooker 2015).

Our data reflect the similarity of insecticide performance across multiple metrics, and the high probability of breaking even economically. This suggests that, instead of the current annual use pattern that govern NST use in maize, they could be readily incorporated into a grower’s insecticide rotation when managing the key pest of maize in Indiana and across much of North America. Alford and Krupke (2017) recently estimated the protection window NSTs provide to the seed and root region of young maize; it is possible that NST concentrations in plant tissues may have intersected with damaging pest populations to generate the stand increases summarized in Fig. 6b. Although no estimates of secondary pest infestations were made during our data collection, wireworm and seedcorn maggot activity periods are well correlated with the high in-plant concentrations of CLO in the seed region (Alford and Krupke 2017). While NSTs can be used for seed protection, their mandatory use is frequently superfluous as wireworms remain an occasional pest across most of the country (Royer et al. 2004) and the likelihood of occasional seedcorn maggot infestations can be predicted based upon incorporation of a green cover crop into the soil (Hammond 1990). A grower should be able to decide if a NST is required based on their individual risk from these seed pests. Finally, while NSTs can provide some control of early season root pests, high concentrations of in plant CLO are not well correlated with CRW phenology (Alford and Krupke 2017). The similarity in root protection (Fig 8a and 8b), coupled with the high probability of breaking even economically (Table 8), indicate that NSTs can easily be incorporated into a grower's insecticide rotation when managing for CRW.

While our study suggests NSTs can be readily incorporated into a grower's insecticide rotation when managing CRW damage within Indiana, Tinsley et al. (2015) suggested rotation of single trait Bt and soil insecticides as a viable CRW management strategy. NSTs were not included in their rotation recommendation as they did not perform as well as in our study. An explanation was not provided for the poor performance of NSTs in Tinsley et al. (2015) but is likely at least partially attributable to historically greater CRW pressure experienced by the states (Illinois and Nebraska) used in their analyses. An estimate of cropping patterns can be derived from satellite data accessible on the USDA-NASS CropScape website (Han et al. 2012). A greater portion of both Illinois and Nebraska farmland favor continuous maize cropping whereas Indiana has a greater adoption of maize/soy rotation. As such it is a possibility that our rotation recommendations can only be applied to areas in which crop production is routinely practiced and CRW pressure is relatively low; this includes much of the corn production area from IN eastward.

While NST are not a primary approach for CRW control, their continual use may eventually render them completely ineffective. The continual maize cropping patterns of the western corn belt led to a reliance on chemical means of CRW control (Pereira et al. 2015) with subsequent resistance to aldrin (Ball and Weekman 1962), methyl parathion (Meinke et al. 1998), carbaryl (Meinke et al. 1998), and bifenthrin (Pereira et al. 2015) by the target pest. A similar fate may be in store for the neonicotinoids, which are not only effectively mandated by maize seed distributors, but an increasing proportion of soybean seed sold in the US is being treated with NSTs as well (Douglas and Tooker 2015). This means that even in areas in which maize is rotated with soy, the field soils where CRW larvae spend their entire life cycle experience a repeated and continual dose of neonicotinoid insecticides. The adaptability of CRW in responding to selection pressures has been demonstrated repeatedly by the rapid evolution of resistance to not only insecticides but also to cultural practices such as crop rotation (both delayed diapause (Krysan et al. 1986) and soybean variant oviposition (Levine and Oloumi-Sadeghi 1996)). Growers are not provided ready access to untreated seed across the US (Douglas and Tooker 2015) despite what is known about CRW resistance potential. Ideally, insecticides would be treated as a limited, finite resource (Sparks and Nauen 2015), and aim to slow the development of resistance by judicious use. I provide data here that suggest that, in the case of NST use relative to CRW, rotation is achievable without exposure to yield losses. Finally, it is worth noting that I am unaware of any areawide resistance monitoring programs for NST with respect to CRW.

3.5 Conclusions

This study demonstrates that, while NSTs are ubiquitous on corn, their contribution to yield and root protection from CRW is no better than established chemistries in Indiana across multiple sites and years. However, unlike the other chemistries in our study, NST use is not an elective choice for US corn producers and is rarely the only approach deployed against insect pests. Our data analysis do not justify the widespread application and use of NSTs in maize partly because they are almost always deployed along with other approaches, such as Bt hybrids (with Bt hybrids accounting for 77% of US maize by area planted in 2017 (USDA-NASS 2017d)) and/or some of the insecticide classes included in our study. A potential, largely unexamined negative aspect of this approach is that the repeated prophylactic use of a single insecticide chemistry is likely to accelerate resistance, partially nullifying benefits associated with NST use. While attempts to maintain the utility of Bt maize and soil insecticides have been made by planting refuges and rotating active ingredients, respectively, neither option is readily available to growers in mitigating the resistance potential of NSTs as they are unable to purchase untreated seed. This lack of choice is difficult to justify, given the apparent redundancy of NST with other available control tactics, coupled with the potential for negative outcomes that typically follow widespread and continuous use of any class of insecticides.

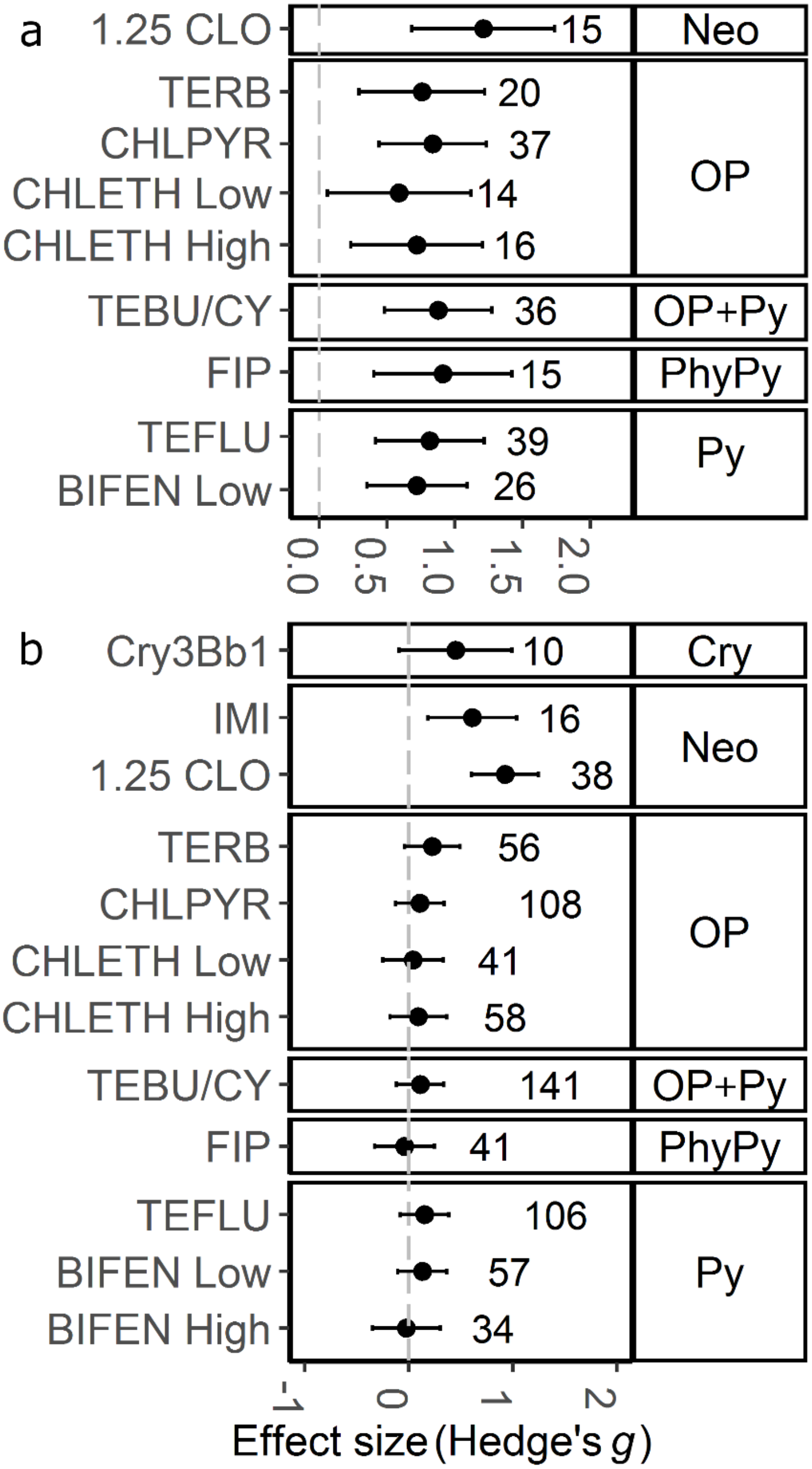


Figure 7. Effect of insecticide on yield (a) and early-season stand counts (b) in comparison to an untreated comparator. The number of replicates used to calculate effect size are adjacent to each bar. 95% confidence intervals are shown

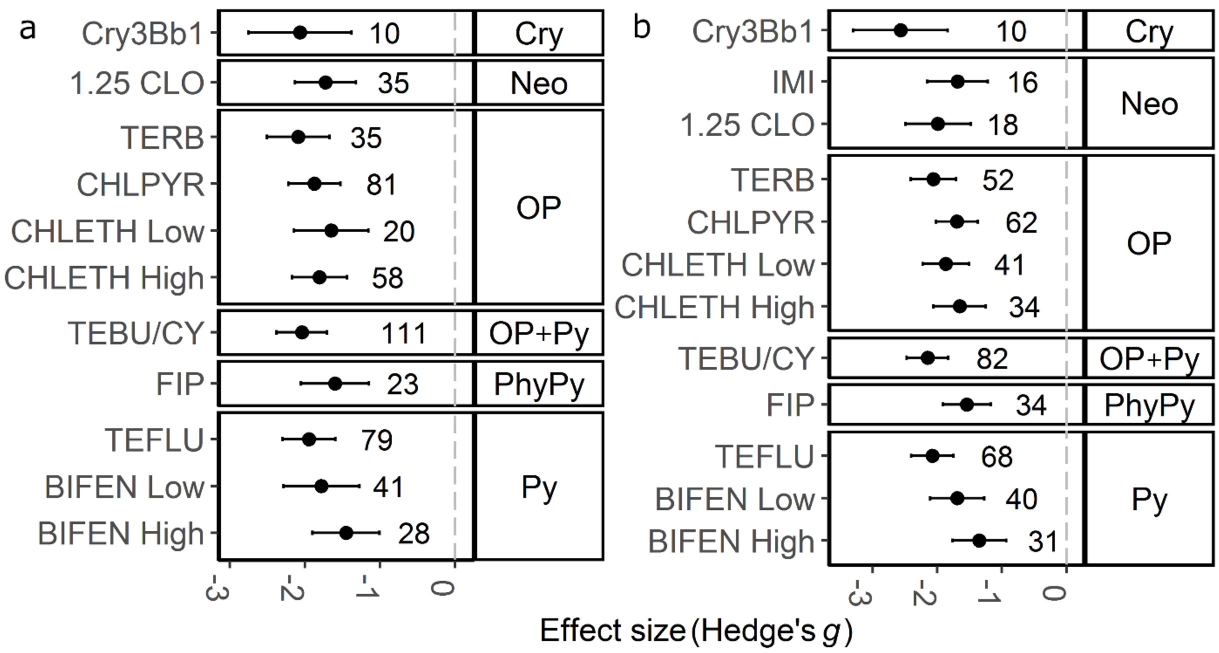


Figure 8. Effect of insecticide on node damage as measured on the Oleson 3-point scale (a) and Iowa 6-point scale (b) in comparison to an untreated comparator. The number of replicates used to calculate effect size are adjacent to each bar. 95% confidence intervals are shown.

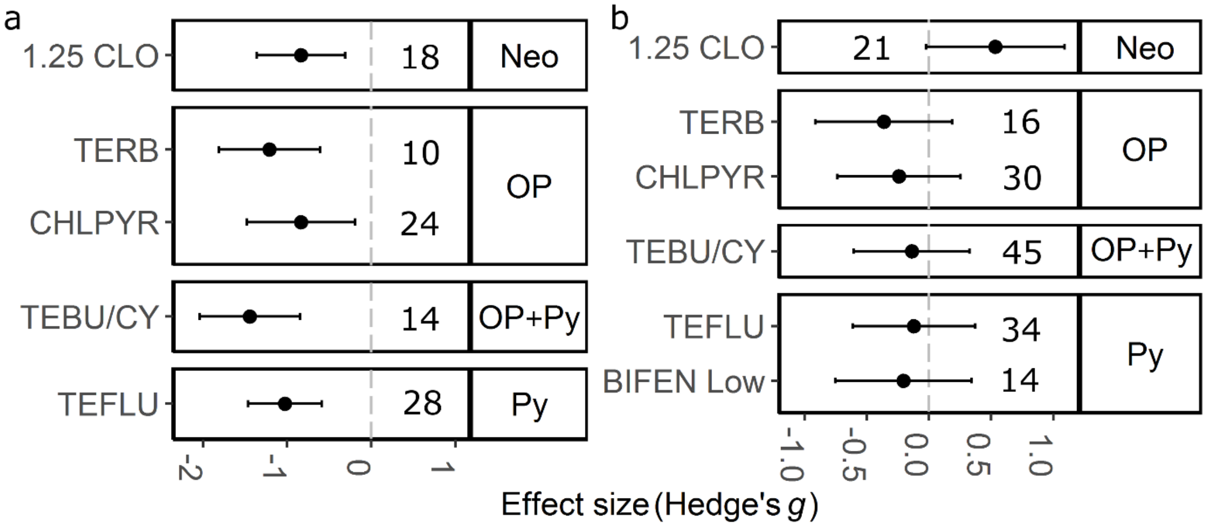


Figure 9. Effect of insecticide on node damage as measured on the Oleson 3-point scale (a) and early season stand counts (b) in comparison to a 0.25 CLO comparator. The number of replicates used to calculate effect size are adjacent to each bar. 95% confidence intervals are shown.

CHAPTER 4: NONTARGET TRANSLOCATION OF CLOTHIANIDIN AT FIELD RELEVANT CONCENTRATIONS AND POTENTIAL TROPHIC LEVEL IMPACTS

4.1 Introduction

The neonicotinoids have become the most widely used insecticide class in the world since becoming commercially available in the early 1990s (Jeschke et al. 2011, Pollack 2011). In many oilseed and grain crops, their main use is as a prophylactic, insurance based, neonicotinoid seed treatment (NST) approach to pest management (Douglas and Tooker 2015) with US adoption rates exceeding >80% in maize by 2011 (Douglas and Tooker 2015, Krupke et al. 2012). Both thiamethoxam (TMX), and its breakdown product clothianidin (CLO), are solely used as NSTs in US maize, whereas foliar and ST options of CLO, TMX, and imidacloprid exist for soy systems. CLO or TMX are applied at rates of 0.25-1.25 mg of compound per maize kernel prior to being sold to the grower. Notably, there is a trend of increasing rates of application in maize per kernel, and this increase is a principal driver of increasing active ingredient/hectare (Douglas and Tooker 2015).

Despite widespread and rapid adoption and use, little has been reported on the translocation of NST from the seed into the target plant in a field setting. Because the target pests live primarily below-ground and are often difficult to detect, one measure of potential efficacy of this approach is quantifying the period of time where insecticides are present in plant tissues. Recent work by Alford and Krupke (2017) documented translocation efficiency of <1.5% of the total active ingredient (AI), in this case CLO, into the target plants in Indiana maize. Translocation efficiency is defined here as the portion of active ingredient that is successfully translocated into plant tissues from the initial active ingredient applied to seeds. One previous study reported maize translocation efficiency from imidacloprid ST to be ~20% (Sur and Stork 2002), although these results are unlikely to be applicable to a field setting as the project was conducted within a greenhouse, and thus was protected from abiotic impacts such as UV photolysis and leaching. Furthermore, Sur and Stork point out that imidacloprid uptake was likely exaggerated due to the amount of soil in each planting box in comparison to root biomass. Imidacloprid is also not commonly used as a seed treatment of maize in today’s marketplace (Douglas and Tooker 2015). The Alford and

Krupke (2017) study thus constitutes our only reference point for the amount of insecticide that could be expected to enter maize plants, leaving 98.5% of the active ingredient unaccounted for. A fraction of the remainder (ca. 2-4%) is lost in the form of planter dust, abraded from seeds during planting and exhausted from planting equipment into the air or down into the soil (Krupke et al. 2012, 2017).

In the environment, both CLO and TMX are hydrolytically stable with high respective solubilities of 0.327g L^{-1} at 20°C and 4.1 g L^{-1} at 25°C. Despite this, they are also relatively stable in soils with the time required to dissipate half the active ingredient (DT_{50}) under field conditions ranging from 277-1386 (US-EPA 2010) and 7.1-92.3 (Hilton et al. 2016) days for CLO and TMX respectively. This is corroborated with published observations documenting residues of both compounds in field soils in our study area of Indiana after two full seasons without neonicotinoid-treated seed (Krupke et al. 2012). As growers do not have easy access to untreated seed (Douglas and Tooker 2015, Alford and Krupke 2018), the current approach leads to a continual and repeated dose of neonicotinoid in the soil. This, in turn, leads to concerns regarding the potential for NSTs to contribute to environmental loading and water contamination via leaching and field runoff (US-EPA 2010). The Groundwater Ubiquity Score (GUS), while not the only metric used in leaching risk assessment, assigns low, medium, and high leaching potentials to GUS values of <1.8, 1.8-2.8, and >2.8 respectively. With the DT_{50} values listed above and respective K_{oc} values of 60 and 68.4 (Pesticide Properties Database 2018), CLO and TMX are assigned "high" GUS values of 5.43-6.98 and 1.84-4.25 respectively. There is no published work explaining this high variability in published DT_{50} values (Goulson 2013) although it is likely due to the inherent properties (clay/sand/silt ratio, pH, etc) of the soil (Bonmatin et al. 2015).

The concerns of environmental loading from NST use appear to have since been justified with increasing reports of neonicotinoids in a range of surface and ground waters with a few of these studies (DeLorenzo et al. 2012, Starner and Goh 2012, Sánchez-Bayo and Hyne 2013, Hladik et al. 2014) suggesting contamination as the direct result of runoff or leaching. In some cases, concentrations have exceeded either acute (Anderson et al. 2013) or chronic (DeLorenzo et al. 2012, Starner and Goh 2012, Sánchez-Bayo and Hyne 2013, Main et al. 2015, Anderson et al. 2013) freshwater invertebrate toxicity benchmarks (US-EPA 2016). The direct role NSTs may play in contributing to environmental contamination has only been studied directly in a few systems (Huseth and Groves 2014, Wettstein et al. 2016). Huseth and Groves (2014) reported a maximum TMX leachate concentration of ~10.7 ± 6.74 ng ml^{-1} recovered from a tension plate lysimeter in potato production with seed treatments however the initial seed treatment concentration was not reported or verified. Direct contamination of subsurface ground water, was also reported with maximum concentrations of CLO and TMX at 0.225 and 0.580 ng ml^{-1} respectively (Huseth and Groves 2014). Wettstein et al. (2016) reported 4-month, flux averaged concentrations of 0.17 and 0.29 ng ml^{-1} for imidacloprid and TMX respectively in sugar beets from initial seed treatments of 0.45 mg imidacloprid and 0.30 mg TMX and seeding rates of 111,222 seeds ha^{-1}.

Table 11 A list of relevant physiochemical properties of common neonicotinoid insecticides. All values taken from the Pesticide Properties Database (2018) with the exception of the Photolytic DT_{50} values which were determined by Lu et al. (2015).

Neonicotinoid	Solubility in H_20 (g l^{-1})	Log K_{ow}	Photolytic DT_{50} (hours)	GUS leaching potential
Acetamiprid	2.95	0.8	26	Low
Clothianidin	0.34	0.905	0.19	High
Imidacloprid	0.610	0.57	0.19	High
Thiacloprid	0.184	1.26	42	Low
Thiamethoxam	4.1	-0.13	0.37	High

While the neonicotinoids, with the exception of thiacloprid and acetamiprid, generally degrade rapidly in clear water (Table 11), a recent experiment conducted in Manitoba, Canada (Lu et al. 2015) demonstrates a potential mechanism by which neonicotinoids can persist within the environment. Vials of TMX treated water were suspended at varying depths within mesocosms representative of Canadian prairie wetlands and photolytic impacts measured. At depths greater than 8 cm, photodegradation of TMX was negligible despite the clear appearance of mesocosm water. The decrease in photodegradation rate was attributed to light screening resulting from organic matter and the surface water. While TMX was the only compound field tested, negligible degradation can likely be expected for other neonicotinoids under similar environmental conditions. Previous laboratory experiments tested the photolytic half-life of acetamiprid, CLO, imidacloprid, TMX, and thiacloprid and determined similar photolytic half-lives for CLO, TMX, and imidacloprid and larger photolytic half-lives for acetamiprid and thiacloprid (Table 11). While this experiment was conducted at a relatively high latitude (50˚N), it demonstrates how increasing depths even in apparently clear water can drastically reduce the photodegradation of environmental neonicotinoids.

The non-target impacts of environmental neonicotinoids have only recently been the subject of increased research effort. Several correlative studies have linked declines in species abundance and diversity to environmental neonicotinoids. These include butterflies (Forister et al. 2016), arthropod natural enemies (Douglas and Tooker 2016), pollinators (Rundlöf et al. 2015), and aquatic macroinvertebrates (Van Dijk et al. 2013), although this latter hypothesis has been contested by Vijver and van den Brink (2014) for ignoring the role of co-occurring insecticides. Another correlative study found insectivorous bird decline was linked to increasing neonicotinoid use. The authors proposed this decrease was not the result of direct toxicity, but rather a result of a trophic cascade via food source reduction (Hallmann et al. 2014). Many of the birds included in the study were obligate insectivores with most of their prey undergoing an aquatic larval stage. The authors of this study postulated that high concentrations of neonicotinoids in water not only negatively impact arthropods, but have potentially wider-reaching cascading effects when viewed in an ecological framework.

Within aquatic systems, neonicotinoid translocation can occur in nontarget macrophytes. and in one such instance CLO and TMX were found at respective concentrations up to 2.01 and 8.44 ng g^{-1} plant tissue in an agricultural wetland system in Saskatchewan CA (Main et al. 2017). While this finding could lead to phytoremediation possibilities, it also serves as an unforeseen route of exposure for non-target phytophagous species. The experimental approach of Main et al. (2017) did not allow for the investigation of neonicotinoid uptake rates, however this information can be extrapolated from laboratory studies. Carvalho et al. (2007) exposed the macrophytes *Lemna minor* (Alismatales, Araceae) and *Lagarosiphon major* (Alismatales, Hydrocharitaceae) to a variety of pesticides and calculated the resulting bioconcentration factor (BCF) at 24, 48, and 72 hours post exposure. The BCF was determined from the following equation:

$$BCF = \frac{Concentration\ in\ plant\ (ng\ g^{-1})}{Concentration\ in\ water\ (ng\ mL^{-1})}$$

While no neonicotinoids were examined in the Carvalho et al. (2007) experiment, in-plant concentrations of all compounds, regardless of physiochemical properties, were found to reach equilibrium by 24 hrs through analysis of BCF values. As such, I may reasonably expect environmental neonicotinoids to rapidly equilibrate within nontarget plant tissue and provide an unconsidered avenue of exposure for macrophyte associated insects.

One such insect is the water lily aphid (*Rhopalosiphum nymphaeae,* Hemiptera, Aphididae), which feeds on numerous macrophyte species. While aquatic plants represent this insect's summer host, water lily aphids are heteroecious, using fruiting trees as overwintering and early spring hosts. In some instances they can become pests of spring plums (Patch 1915) although they are much more well known as being a pest of cultivated aquatic plants. This species is capable of transmitting abaca mosaic, cabbage black ringspot, cauliflower mosaic, cucumber mosaic, and onion yellow dwarf viruses (Center et al. 2002). The water lily aphid's aquatic plant host range is broad and includes, but is not limited to arrowheads (*Sagittaria* spp., Alismataceae), duckweeds (*Lemna* spp. Araceae), spatterdock (*Nuphar luteum* (L.) Sibth. & Sm. Nymphaeaceae), waterlettuce (*Pistia stratiotes* (L.) Araceae), and watermilfoils (*Myriophyllum* spp. Haloragaceae) (Center et al. 2002).

Considering the increasing documentation of instances of runoff and leaching of neonicotinoids, there is a clear need to describe more clearly: 1) the rate at which neonicotinoids leave crop seeds and enter the aquatic environment, and 2) the potential effects of the resulting aqueous residues on aquatic ecosystems. Furthermore, a growing body of literature supports the hypothesis that environmental neonicotinoids negatively impact non-target organisms either through direct toxicity or via contamination or reduction of food and water sources. The goal of the research described here was two-fold: first, to quantify the leaching potential of NST throughout the growing season in Indiana maize. Using concentrations documented by this objective and from existing published literature, this work was then expanded upon with manipulative laboratory experiments using gibbous duckweed (*Lemna gibba,* Alismatales, Araceae) and the water lily aphid as model organisms in order to document both translocation dynamics into non-target aquatic plant tissues and demonstrate potential impacts upon higher trophic levels. Both the gibbous duckweed and water lily aphid were partly chosen due to their history as model organisms with published propagation and rearing methods (Hance et al. 1994, Brain and Solomon 2007). Moreover, the relationship between aphid and duckweed is not entirely removed from what would occur in a more natural environment. A review of duckweed associated insects reported that 39 insects utilized duckweed mats as shelter, food, or both (Scotland 1940). Furthermore the eutrophic, nutrient rich waters where duckweed is most common are typified by ditches adjacent to tile drained fields and provide a potential route for nontarget translocation in these plants. Finally, it is worth noting that because duckweeds are known to create dense mats over the surface of the water body they inhabit, they can dramatically reduce light penetration (Zirschky and Reed 1988) and potentially extending the half-life of these highly photolytic compounds.

4.2 Materials and Methods

4.2.1 Experimental site, setup, and sampling

Planting of maize hybrid 5337SX (Becks, Atlanta IN, USA, RM: 103 days) took place at the Purdue Water Quality Field Station (WQFS) (40.4903083°, -86.9952139°) on May 23/2016. The soil at this site is classified as a Drummer silty clay loam (Ziegler and Wolf 1998) and received yearly precipitation of 880.74 ± 44.83 mm, on average, from 2005-15. Maize seed was treated with 1.25 mg CLO (Bayer Crop Science, Monheim am Rhein, Germany) per kernel and expressed the Bt toxins cry2Ab2, cry1A.105, cry1Fa2 for lepidopteran (i.e. above-ground) pest management and toxins cry3Bb1, cry34Ab1, and cry35Ab1 for corn rootworm pest management. Soy variety P34T07 (DuPont Pioneer, Johnston, Iowa, USA) was used for soy plots, and while NST are available, NST free soybean seed was used. Maize and soybeans were planted at 74,130 and 642,473 seeds hectare^{-1}.

The WQFS is comprised of 48 small plots and 8 large plots. Small plots measure 10 x 48.5 m, and contain a clay box lysimeter (10 x 24 m) with walls that extend down to the glacial till layer (1.5 m). In contrast, large plots are 60 x 48.5 m, and utilize guard tiles to maintain 10, 20, and 30 m tile spacing. Each clay box lysimeter is tile line-drained (0.1 m diameter) and is placed 0.9 m below the soil surface. Each plot's tile drainage line is perforated only within its respective clay box to limit contamination between plots. Drainage lines run to collection huts, which protect samples from both rainfall and sunlight, and drain leachate directly into tile-specific tipping buckets, which deposit a water subsample (~10 ml) into a 20 L collection bucket every other tip (Fig. 10). Samples were collected daily, when flow occurred, so that any pesticide present in the sample represented the average concentration over the previous ~24 hr period. Samples were stored at -20°C until further processing. Data were only collected from rainfall events when more than 35 tiles flowed irrespective of large/small plot designation. This decision was made to provide sufficient data for a given rainfall and tile flow event as both the period of time between precipitation and resultant tile flow, as well as flow duration, were variable between plots. This was likely due to a variety of factors including, but not limited to, crop history, tillage practices, and condition of tile drainage system (root damage to tiles, blockage by wildlife, etc.).

Table 12. Planting regimes in plots sampled at the WQFS in 2016, including abbreviation (Abbr.) and relevant agronomic practices.

Crop	Abbr.	Residue removal?	Spring disk?	Fertilizer	Starter
Prairie grass	PG	Y	N	None	None
Miscanthus	MIS	Y	N	50 lb Urea/acre + Agrotain broadcast	None
Switchgrass	SG	Y	N	50 lb Urea/acre + Agrotain broadcast	None
Sorghum	SOR	Y	Y	160 lb N/acre preplant	None
Cont. Maize	CM1	Y	N	160 lb N/acre preplant	19/17/0
Cont. Maize	CM2	N	Y	None	19/17/0
Cont. Maize	CM3	N	Y	None	19/17/0
Cont. Maize	CML	N	Y	160 lb N/acre preplant	None
Rotate Maize/Soy	MS1	N	Y	140 lb N/acre preplant	19/17/0
Rotate Maize/Soy	MS2	N	Y	120 lb N/acre preplant	19/17/0
Rotate Soy/Maize	SM1	N	Y	None	None
Rotate Soy/Maize	SM2	N	Y	None	None
Rotate Soy/Maize	SM3	N	Y	None	19/17/0

Of the 48 small plots at the WQFS, 20 plots were planted with maize, 12 with soybean, and 4 plots each for the remaining treatments (sorghum, prairie grass, miscanthus, switchgrass). The 6 large plots were planted with continuous maize. The prairie grass plots have been comprised of native Indiana grasses since 1996 and served as a negative control in which little or no neonicotinoid insecticide residues were expected in leachate samples. A complete description of treatments is presented in Table 12. Plots received no irrigation beyond rainfall.

4.2.2 Processing of water samples

Solid phase extraction was used to prepare water samples for analysis with HPLC. First, Oasis HLB 12 cc filter cartridges (500 mg sorbent, 60 µm particle size, Waters Milford Massachusetts) were attached to a Preppy™ 12-port vacuum manifold (Sigma-Aldrich St. Louis Missouri) and conditioned with 5 ml of HPLC grade acetonitrile (Sigma-Aldrich St. Louis Missouri) followed by water equilibration (5 ml). Sixty ml of a water sample was then passed through the conditioned cartridge, eluted twice with 1.5 ml of HPLC grade acetonitrile, and evaporated within a single tube in a speedvac. Samples were re-suspended in 100 µL ACN and analyzed with an Agilent 1200 Rapid Resolution liquid chromatography (LC) system coupled to an Agilent 6460 series QQQ mass spectrometer (MS) (Santa Clara, CA, USA). Machine settings for the MS are reported in Appendix A. Calibration curves of 0.001-10 ng ml^{-1} were used to quantify CLO and TMX concentrations in WQFS samples and an internal standard was used in the quantification of CLO in all duckweed samples. The LOD of duckweed samples was 1-10 ppt.

4.2.3 Analysis of WQFS data

Tiles were variable in both how long it took to initiate flow, and overall flow duration per precipitation event. For example, some tiles would flow 1-2 days, whereas others would flow for 3-5 days following the same precipitation event. These discrepancies are due to a variety of likely factors including differences in plot history, tillage practices and tile drain condition. To minimize the effect of differential flow for data comparison purposes, samples were matched up by the peak CLO concentration per precipitation event instead of by sampling date.

To further account for variable tile flow and maximize data available for analysis, three prospective groupings were created based upon initial seed treatment rate and plot history. These groupings were maize plots (Table 12: CM1, CM2, CM3, CML, MS1, MS2) which were treated with 1.25 mg CLO kernel^{-1}, NST free soybean plots (Table 12: SM1, SM2, SM3), and untreated control plots (Table 12: MIS, PG, SOR, SG). As each field plot has undergone varying crop rotations and combinations of starter/fertilizer over the past decade, each prospective grouping was analyzed with a multivariate approach to repeated measures. Fixed main effects included treatment, sampling date, and a multivariate treatment*sampling date interaction effect as predictors of CLO concentration within leachate. The time*treatment interaction effect was assessed for each proposed grouping, with an insignificant result indicating the suitability of creating a new treatment group from each proposed grouping (Maize, Soy, Control). Levene's test was used to assess homogeneity of variances in all multivariate models.

Because the time*treatment effect was insignificant for all three models (Table 13), each plot treatment was reclassified into a new Maize, Soy, or Control treatment. These new treatment groupings were then analyzed with the same multivariate approach to repeated measures model as described above, but results were followed by univariate analysis by sampling event, to compare CLO concentrations in treated maize plots to untreated soy and control plots. All statistical models within this section were analyzed with Statistica (Version 13.3) (TIBCO Software Inc. 2017).

4.2.4 Maintenance of duckweed and aphid cultures

The G3 strain of *L. gibba* was maintained in axenic culture as described by Brian and Solomon (2007). In brief, axenic fronds of *L. gibba* were transferred within a laminar flow hood to autoclaved 2800 ml culture flasks, containing 1000 ml of fluid fortified with half strength Hutner's growth media (Brian and Solomon 2007) and stoppered with a cloth plug. Initial *L. gibba* stocks were provided by Paul Fourounjian at Rutgers University. Newly established colonies were allowed to grow at 25˚C, under constant light measured at 43,000 lx. Colonies grew until they covered the surface of the 2800 ml flask. At that point, a portion of duckweed was removed and transferred to a new culture flask as before and the remaining duckweed was used for experimentation or as food/substrate for aphid colonies.

Colonies of *R. nymphaeae* were maintained in 8 L aquaria (15 x 30 x 20 cm) at room temperature under constant light measured at 126,000 lx. Tanks were filled with de-ionized water fortified with half strength Hutner's media and used to propagate *L. gibba* from opened culture flasks. Aphids were initially collected from duckweed (*Lemna* spp.) located at the Purdue Wildlife Area (40.452293°, -87.054987°) in autumn 2016 and maintained on laboratory cultures of *L. gibba* after collection. Tanks were cleaned and water/nutrients replaced as needed when duckweed populations crashed due to aphid overfeeding, or algal growth began outcompeting duckweed fronds for growing space. Aquaria were also supplemented with aeration pumps to provide gentle water movement. This reduced competition from various biofilm-creating microorganisms, which limited the lateral growth of duckweed colonies.

4.2.5 Translocation dynamics of duckweed

The uptake dynamics of aqueous CLO was investigated with the use of *L. gibba*. Selected test concentrations were well below the no-observed-effect concentration of 90,200 ng ml^{-1} (Finnegan et al. 2017). As the environmental chamber dehumidifier was non-operational, addition of water-filled tanks to the environmental chamber would increase relative humidity. To determine if this change in humidity would impact the rate of CLO uptake and ultimate in-plant CLO concentration, two test humidities were used (60 and 80% relative humidity). These humidities were selected as they correspond to both the minimum and maximum humidity observed during the aphid LC_{50} trials (next section). Each experimental tank consisted of exposing ~95 cm^2 of duckweed to three CLO concentrations (0, 2, or 10 ng ml^{-1}) in a 4 L aquarium (20.5 x 11.5 x 18.5 cm), at 25˚C, under constant light. The 2 and 10 ng CLO ml^{-1} tanks were both replicated three times, whereas the control tank was only replicated once. Each humidity trial was conducted a single time with full replication of the 2 and 10 ng CLO ml^{-1} test concentrations. The differing proportions between a 4 L aquarium and 2,800 ml culture flask, led to duckweeds being closer to the growth lights with a more field relevant light intensity of 95,000 lx. For comparison, solar noon in West Lafayette Indiana produces a lux ~ 110,000. Preliminary trials found CLO concentrations reached equilibrium within plant tissues within 8 h under these conditions and all subsequent translocation trials were carried out over a 12 h time span. Every 2 h following duckweed introduction, the humidity was recorded. Every 4 h post-exposure, ~0.5-1 g of duckweed were removed from each experimental unit, quickly dried between two paper towels to remove excess water, fresh weight recorded, and briefly stored in a 7 ml homogenization tube. After duckweed was sampled from each experimental unit, plants were homogenized and processed with a modified QuECHeRs protocol as described in Appendix A. A 50 ml water sample was also collected prior to duckweed introduction to confirm concentrations. Quantification was performed using Liquid Chromatography tandem Mass Spectrometry (LC-MS) with a LOD of 0.1 ng g^{-1}. Following quantification, the fresh weight bioconcentration factor (BCF) was calculated as described above (Carvalho et al. 2007). A multivariate approach to repeated measures, followed by univariate results by sampling event was used to compare BCF as a function of relative humidity in Statistica (Version 13.3) (TIBCO Software Inc. 2017). Separate models were run for each concentration (2 and 10 ng CLO ml^{-1})

4.2.6 Aphid LC_{50} trials with contaminated duckweed

The water lily aphid (*Rhopalosiphum nymphaeae,* Linnaeus) was exposed to contaminated duckweed in a series of experiments to demonstrate potential trophic impacts of neonicotinoid contaminated plant tissue. Each trial was conducted at the same tank size, temperature, and light duration and intensity as in the translocation dynamics experiment. Due to a non-operational dehumidifier in the environmental chamber, relative humidity ranged from 61-81%. Each experimental replication consisted of one control tank, in which no CLO was added to the water, and several experimental tanks with varying concentrations of CLO. Due to variability in CLO stock solutions and within plant tissues, a variety of target concentrations were tested ranging from 0-33.99 ng ml^{-1}. At the start of each trial, ~ 32 cm^2 duckweed were introduced in a 4 L tank. Duckweed was allowed to equilibrate and grow for the first 48 h to both expand the surface area upon which aphids could walk, and provide sufficient plant material for future samples. After 48 h, 15 apterous aphids were added to a floating plastic petri dish (60 mm diameter x 15 mm in height) and left to disperse and feed. This approach greatly reduced the risk of handling mortality in comparison to transferring aphids to individual thalli. Water samples (50 ml) were collected immediately prior to addition of duckweed (0 h), after addition of aphids (48 h), and at the end of the trial (96 h), in order to both ensure and monitor how target concentrations changed over time. Similarly ~ 0.33-0.5 g of duckweed was collected prior to aphid addition (48 h) and removal (96 h), and was processed as in the translocation experiment. Duckweed samples allowed me to ensure equilibration had been reached and to determine what CLO concentration aphids were initially exposed to. Aphids were collected at 96 h and mortality of apterous adults recorded. This species of aphid is parthenogenic, and data were not collected on any nymphs resulting from live birth during the 96 h experimental runs.

Both water and duckweed samples were analyzed as in the translocation dynamics experiment, and a BCF was calculated as well. Aphid results were analyzed with PROC probit in SAS (SAS Institute 2018) to determine the LC_{50}. PROC probit uses Abbott's formula to correct treatment mortality by using the proportion observed mortality in control tanks (Abbott 1925). As the aphids in our oral LC_{50} experiments were also potentially exposed to CLO through contact with contaminated water this trial is inherently confounded by this variable, as would be the case in the aphids' natural setting. In any event, the oral LC_{50} is typically lower than contact LC_{50} and thus, while contact with contaminated water may contribute to aphid mortality, feeding on contaminated plant tissue is likely to be a more important driver. Finally, while the water lily aphid can break the surface tension to feed on submerged portions of plants (Scotland 1940), this behavior was not observed in any experimental tank or aphid colony tank. Aphids were observed walking on the water surface when not feeding however.

4.3 Results

4.3.1 WQFS leachate concentrations

The highest average CLO concentration in maize leachate across the season (3.48 ± 0.27 ng ml^{-1}; n=6), corresponded with the first rainfall event (6/23/16), after planting (5/23/16), that resulted in >35 tiles flowing (Fig. 11). The next precipitation event resulting in flow from >35 tiles occurred on 7/18/16 with an average acute CLO concentration of 1.55 ± 0.27 ng ml^{-1} (n=6) in maize plots. Drought conditions (cumulative rainfall of 281 mm from 7/20/16 to 10/31/16) prevented the collection of any additional water samples throughout the rest of the growing season (USDA-NASS 2016b). The next three rainfall events resulting in flow of >35 tiles were 12/26/17, 1/17/17, and 3/31/17, with respective CLO concentrations of 0.10 ± 0.01 (n=6), 0.08 ± 0.02 (n=6), and 0.6 ± 0.01 ng ml^{-1} (n=5). CLO concentrations in the switchgrass, prairie, Miscanthus, and sorghum plots never exceeded 0.1 ng ml^{-1} at any point in the sampling range indicating limited CLO contamination between tiles.

The varying combinations of starter, fertilizer, and tile history were determined not to be significant predictors of CLO concentration in leachate, within a given crop group (Table 13). As such, all CLO treated maize plots (Table 12: CM1, CM2, CM3, CML, MS1, MS2), all soybean plots (Table 12: SM1, SM2, SM3), and untreated control plots (Table 12: MIS, PG, SOR, SG), were grouped together for the CLO analysis. A significant multivariate treatment*time was recorded in the CLO model indicating CLO concentrations were different across the sampling period (Table 14). Univariate treatment effects by sampling day in the CLO model were highly significant on the first and second sampling event following planting (Table 15).

4.3.2 Translocation dynamics of duckweed

At the 2 ng ml^{-1} concentration, only time was significant (Time: Wilks , $F_{2,3}$ = 16.46, P = 0.024; Time*Humidity: Wilks, $F_{2,3}$ = 2.64, P = 0.218). At the 10 ng ml^{-1} concentration, neither variable was significant (Time: Wilks , $F_{2,3} = 7.61$, $P = 0.067$; Time*Humidity: Wilks, $F_{2,3} = 9.03$, $P = 0.04$). The, univariate results found significant differences at all sampling points at the 2 ng ml^{-1} concentration, but only at the 4, and 8 h time point at the 10 ng ml^{-1} concentration indicating similar concentrations at the 12 h time point (Fig. 12, Table 17). The average humidity for the high and low humidity trial were 80.71 ± 0.51% (n=14) and 60.5 ± 0.27% (n=7). Measured CLO concentrations within experimental aquaria were 158.74 ± 31.31% (n=12) of target concentrations of 2 and 10 ng CLO ml^{-1} across all trials and humidities.

Table 13. F-statistics and estimated degrees of freedom (df) for the multivariate repeated-measures ANOVA model assessing the suitability of grouping treatments by initial seed treatment rate for CLO. Significant results are denoted by an * with $^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

Group	Factor	df	F-statistic
Maize	Time	4, 6	41.02^{***}
	Time*Treatment	20, 20.8	0.03
Soy	Time	5, 1	5.33
	Time*Treatment	10, 2	0.78
Control	Time	4, 1	0.24
	Time*Treatment	12, 2.9	0.09

Table 14. F-values and estimated degrees of freedom (df) for the multivariate repeated-measures ANOVA model assessing CLO concentrations in leachate as a result of initial seed treatment. Significant results are denoted by an * with $^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

Grouping	Factor	df	F-value
CLO	Time	5, 13	15.36^{***}
	Time*Treatment	10, 26	8.37^{***}

Table 15. Univariate F-values and degrees of freedom (df) generated following the univariate repeated-measures ANOVA model describing the influence of initial NST (Compound) on neonicotinoid concentration in leachate following planting. Significant results are denoted by an * with $^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

Compound	df	5/11/16	6/23/16	7/18/16	12/26/16	1/17/16	3/31/17
CLO	2, 17	1.93	63.77^{***}	10.60^{**}	1.65	1.45	1.44

Table 16. The mean BCF and SE of *L. gibba* grown in half-strength Hutner's media fortified with CLO at 2 and 10 ng ml^{-1} concentrations. Within a given time point and target concentration, means followed by the same letter denote statistical similarity as determined by t-test ($P = 0.05$).

Target Conc.	RH	4 h	8 h	12 h
2 ng ml^{-1}	60%	1.00 ± 0.03 (n=3) a	1.19 ± 0.07 (n=3) a	1.23 ± 0.07 (n=3) a
2 ng ml^{-1}	80%	0.68 ± 0.03 (n=3) b	0.89 ± 0.04 (n=3) b	0.74 ± 0.05 (n=3) b
10 ng ml^{-1}	60%	0.71 ± 0.06 (n=3) a	0.66 ± 0.06 (n=3) a	0.78 ± 0.11 (n=3) a
10 ng ml^{-1}	80%	0.43 ± 0.01 (n=3) b	0.48 ± 0.02 (n=3) b	0.54 ± 0.04 (n=3) a

Table 17. Univariate F-values and degrees of freedom (df) generated following the univariate repeated-measures ANOVA model describing the influence of humidity on the BCF of CLO and *L. gibba* at two different CLO concentrations. Significant results are denoted by an * with $^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

	df	4 h	8 h	12 h
2 ng ml^{-1}	1, 4	54.11**	15.60*	33.56**
10 ng ml^{-1}	1, 4	22.54**	8.92*	4.34

4.3.3 Aphid LC$_{50}$ trials with contaminated duckweed

Measured CLO concentrations within water were 89.49 ± 1% (n=27) of target test concentrations, with a corresponding BCF of 58.93 ± 2.86% (n=27) for all experimental tanks. The relative humidity ranged from 61-81% over all 7 experimental trials and 3 experimental tanks were not included in the analysis due to CLO contamination during the homogenization step. A total of 397 aphids were exposed to CLO contaminated duckweed and found to have a probit-estimated (95% fiducial limits) LC$_{50}$ and LC$_{99}$ of 8.61 (6.23-9.76) and 20.46 (15.25-70.88) ng CLO g plant tissue^{-1} respectively (Fig. 13). Due to a significant result following a goodness-of-fit test ($\chi^2 = 58.88$, df=25, $P < 0.0001$), a t-value of 2.06 was used in the calculation of 95% fiducial limits. Despite this, the lowest concentration at which 100% mortality was observed was at 16.78 ng CLO g plant tissue^{-1}. The average control mortality across all trials was 3.8%.

4.4 Discussion

The work reported here provides documentation of the timing and magnitude of the potential contribution of NSTs to waterway contamination. The maximum CLO concentrations we report here of 3.48 ng ml^{-1} are approximately an order of magnitude greater than CLO concentrations previously reported in streams/rivers (Sánchez-Bayo and Hyne 2013: 0.42 ng ml^{-1}; Hladik et al 2014: 0.257 ng ml^{-1}; Hladik et al. 2018: 0.226 ng ml^{-1}) and prairie wetlands (Main et al. 2015: 0.142 and 0.059 ng ml^{-1} for 2012 and 2013 respectively). This is not surprising, as our results present a direct and undiluted measure of neonicotinoid leachate and reinforces the contribution of NST to environmental contamination. In addition, collection directly from tiles beneath ground level is not subject to photodegradation. These are likely to be the two principal factors influencing neonicotinoid concentrations in our samples and those collected by other researchers. Furthermore, this work demonstrates that NST concentrations within leachate are greatest at precipitation events that follow planting and largely conform to a first-order decay pattern of initially high concentrations, with a rapid and drastic concentration decrease as the growing season progresses. While the drought conditions of 2016 limited our access to rainfall data, a similar first-order decay pattern was also reported by Wettstein et al. (2016). This result is expected as rainfall increases surface water contamination potential (Chiovarou and Siewicki 2008), and as cumulative rainfall increases, less active ingredient is likely left in the soil due to a combination of plant uptake, leaching, and breakdown.

Using a multivariate approach to repeated measures, post-planting concentrations of CLO in leachate samples were statistically similar to untreated plots by the 12/26/16 rainfall event. Concentrations of CLO peaked at the first rainfall event and decreased throughout the year (Fig. 11). Unfortunately, drought conditions after the 7/18/16 rainfall event limited the resolution for detecting further changes in concentrations throughout the latter part of the season. Additionally, while the concentrations in the leachate of the untreated plots (switchgrass, prairie, miscanthus, and sorghum) were extremely low for CLO (<0.1 ng ml^{-1}), concentrations were not 0. Subsurface lateral flow has been reported as a contamination pathway in the transport of neonicotinoids to nontarget soil (Bótias et al. 2015, Long and Krupke 2016). While the clay box lysimeters likely prevented the majority of inter-tile subsurface flow, maize seed is planted in the first few cm of the soil profile and the clay box lysimeters do not extend to the soil surface with walls starting ~122 cm below the soil surface. Given the high solubility of NST, this is a likely explanation for

CLO presence in non-maize plots. Another, complementary, explanation of inter-tile contamination is the occasional flooding that occurs throughout the year at the WQFS. This can result in floodwater transferring soil bound NST from one tile, and depositing it to another during percolation. These factors in combination with the high soil DT_{50} of CLO may explain why CLO is present in soybean plots however the reported concentrations cannot wholly be attributed to inter-tile contamination or carry over from the previous years NST maize planting. Soybean plots had an overall higher CLO concentration within leachate in comparison to untreated plots alone. If carry over from the previous years planting was the predominant factor, preplant CLO concentrations would be similar to post plant CLO concentrations. If inter-tile contamination was the predominant factor, control plots would have similar CLO concentrations within leachate to soybean plots. This latter explanation however assumes inter-tile contamination to be equal across plots. Any variety of factors such as changes in elevation, minor changes in soil composition, and even placement of plots make this unlikely. While the ultimate cause of higher than expected CLO concentrations within soy plots was beyond the scope of this experiment, additional studies looking at how NST interact with the surrounding soil may provide an explanation.

My experiments investigating the uptake dynamics of CLO into duckweed found in-plant concentrations rapidly increased within 4 h of exposure to CLO contaminated water. Furthermore, significant differences at most sampling points at both tested concentrations confirm the role of humidity in mediating in-plant CLO concentrations. This rapid uptake and consistent BCF coincides with the findings of Carvalho et al. (2007), who reported equilibration occurred by 24 h in *L. minor* when exposed to a variety of pesticides with varied physiochemical properties. The role humidity plays in duckweed translocation dynamics was investigated by subsequent experiments in order to account for any impacts variable humidity may have had on the aphid LC_{50} trials. Due to a non-operational dehumidifier, addition of water-filled tanks to the environmental chamber would increase relative humidity and potentially impact the CLO concentration aphids were exposed to through the consumption of contaminated duckweed. Despite the humidity-mediated uptake differences at most sampling points within the uptake trials, any humidity mediated impacts to in-plant CLO concentrations appear to be minor within the scope of my aphid LC_{50} trials as the average BCF across all trials was 58.93 ± 2.86% (n=27). The consistency of BCF in the aphid trials may be due to the 2 d period while duckweed was allowed to grow, uptake CLO, and cover the water surface.

While the protocols described here were designed to limit the impacts of photolysis and dilution, the dense mats of plant tissue that duckweeds and other floating macrophytes create in agricultural ponds and lakes similarly reduce light penetration, likely reducing the impact of photolysis. This plant-associated reduction in light penetration, in combination with the overall decrease in photodegradation rate resultant from a compound's position within the water column (Lu et al. 2015), provide a mechanism for these otherwise photolytic compounds to persist within the environment. The nontarget translocation of aquatic neonicotinoids has only been reported in submerged and rooted macrophytes (Main et al. 2017), however future environmental monitoring studies would benefit from the targeted sampling of floating macrophytes such as the duckweeds. While my results demonstrate the rapidity with which aqueous CLO is translocated into *L. gibba*, the translocation mechanism is relatively unknown. The uptake of nutrients, and presumably CLO as well, is still poorly understood within the Lemnaceae (Fang et al. 2007). Both the roots and frond of *L. minor* have nutrient uptake abilities, however the root region appears to be more significant contributor in N-depleted plants (Cedergreen and Madsen 2002). Additionally, organic chemicals with an octanol-water partition coefficient (log K_{ow}) between 0.5 and 3 are considered hydrophobic compounds that are able to move through the lipid bilayer of membranes but still water-soluble enough to travel into cell fluids (Cedergreen et al. 2005). The log K_{ow} represents the 1-octanol/water partition coefficient and is used as a measure of lipophilicity. A combination of lipid bilayer penetration and uptake by the plant is likely to have occurred in this study as CLO has a log K_{ow} of 0.905. Independent of the mechanism(s) at play, my research and previous work demonstrate that translocation of CLO into nontarget vegetation within the environment is likely to occur quickly, and provides a route of nontarget exposure to organisms that utilize or feed on contaminated vegetation.

The maximum CLO concentrations we report here are an approximate order of magnitude higher than other published literature (Sánchez-Bayo and Hyne 2013, Hladik et al 2014, Main et al. 2015, Hladik et al. 2018). Despite this, my highest leachate concentration (3.48 ng ml^{-1}) was less than half of the LC_{50} (8.71 ng g plant $tissue^{-1}$) of *R. nymphaeae* and corresponds to a probit-estimated mortality probability of <1. Using mortality as my only metric, no toxic effects were observed in *R. nymphaeae* at field relevant CLO concentrations. However, toxic effects have been elicited on both organismal and community scales with other neonicotinoids. One such experiment found aquatic predator mortality increased by 52% when exposed to 352 ng CLO ml^{-1} over 21 d, in comparison to the 0.6 ng CLO ml^{-1} treatment (Miles et al. 2017). While 352 ng CLO ml^{-1} is ~ 2 orders of magnitude greater that reported in literature, this study demonstrated differential toxicity between aquatic herbivores and predators with the latter being more affected at lower concentrations. Another microcosm experiment (Basley and Goulson 2018) investigated the colonization of water bodies as a result of neonicotinoid contamination. Mesocosms were created from 400 g of loamy soil and 10 L of water, contaminated at several concentrations (CLO and TMX at 0.1, 1, 3, 7, 10, 15 ng ml^{-1}), and left open to the environment. The three most prominent colonizing groups were chironomids (midges), ostracods (crustaceans), and *Culex* mosquitoes. Of these three, chironomids and ostracods were the most negatively impacted by increasing neonicotinoid concentrations whereas *Culex* larvae were relatively unaffected. Finally another long-term (7 month) mesocosm experiment utilized closed steams to investigate the impacts of a single application of thiacloprid (0.1, 3.2, and 100 ng ml^{-1}) (Beketov et al. 2008). Of the 21 surveyed species, 11 were considered long-lived (<1 generation per year) and were mostly comprised of representatives from Odonata, Plecoptera and Trichoptera. Ten species were considered short-lived (>1 generation per year) and were comprised mainly of Diptera, Ephemeroptera, Isopoda, and Oligochaeta. Following the application of thiacloprid, short-living species recovered after 10 weeks whereas long-living species recovered at 7 months, the end of the experiment at both the 3.2 and 100 ng ml^{-1} level. These three mesocosm experiments, in combination with the correlative field studies mentioned earlier (Van Dijk et al. 2013, Hallmann et al. 2014), have allowed researchers to better describe and predict how neonicotinoid contamination of water bodies can lead to nontarget impacts in not just aquatic but terrestrial environments on a community scale.

Previous research has shown that a small fraction of NST (<1.5%) is actually translocated into maize plant tissue, with the remaining ~98% presumably entering the environment via multiple pathways (Alford and Krupke 2017). While CLO ST perform just as well in terms of yield protection in maize as previous insecticide classes within Indiana and areas in which crop rotation is practiced (Alford and Krupke 2018), they are much more likely to leave the field, due to their increased solubility coupled with a high DT_{50}. They also are largely redundant as a consequence of > 90% of corn hybrids expressing Bt toxins targeting key pests (Alford and Krupke 2018). My study found neonicotinoid concentrations within leachate to persist up to 2 months post plant however a drought limited any extrapolation between 2 and 7 months post plant, at which treated plots were statistically similar to untreated plots. Once within an aquatic system, we can expect nontarget macrophytic translocation to be both rapid and to reach equilibrium within plant tissues by 12-24 h. While my laboratory studies only used *L. gibba*, these results are likely pertinent to other duckweeds and free-floating/submerged macrophytes. Additional studies investigating CLO depuration within macrophytes should be conducted to assess the stability of CLO, and other NST within contaminated plant life. Results from such an experiment will allow a greater understanding of neonicotinoid persistence in nontarget plant tissue, and may expand environmental monitoring opportunities. Finally, the LC_{50} experiment I conducted here, can be expanded upon to identify any confounding effects of NST contamination in problematic macrophytes, for which an insect based biological control program has been implemented.

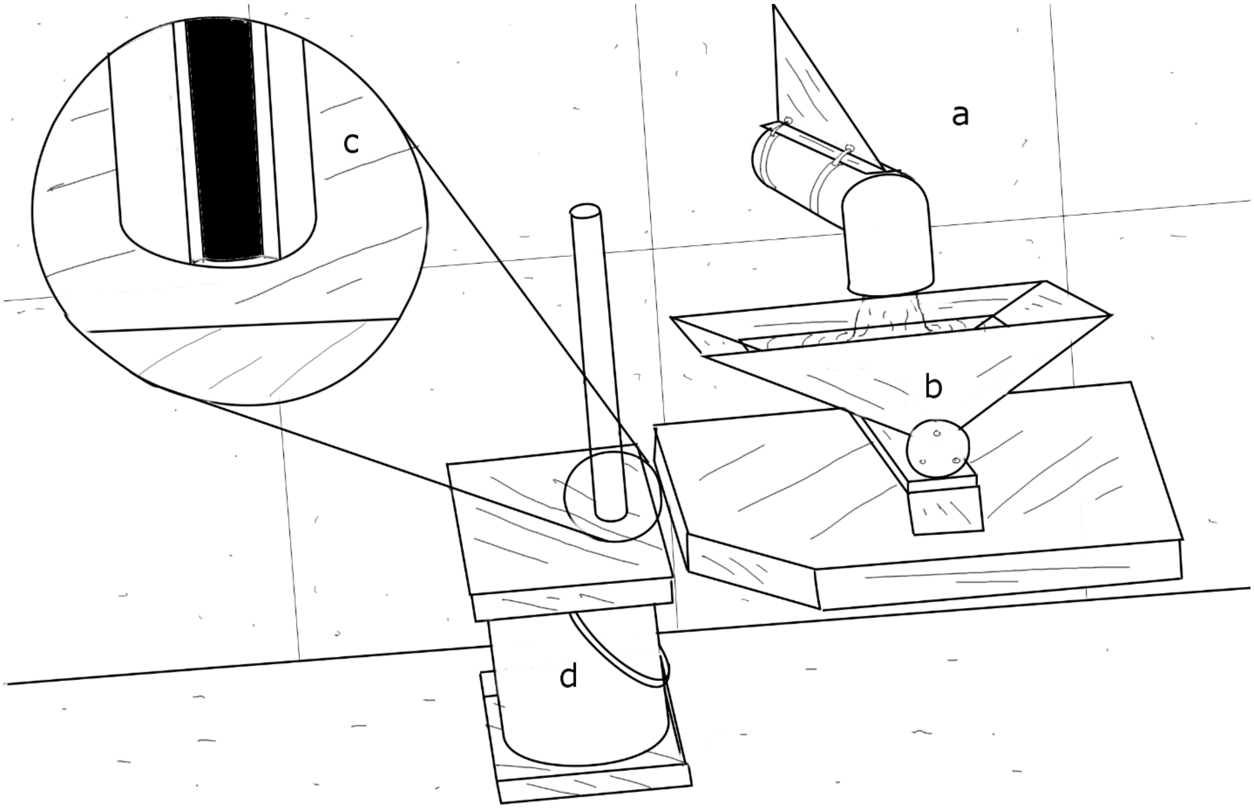


Figure 10. The WQFS sampling system. Plot specific tile leachate flows from the intake pipe (a), where it is collected in the tipping bucket (b). Every other tip, approximately 10 ml of leachate goes through the collection bucket intake (c), where it rests in the collection bucket (d) until sampling.

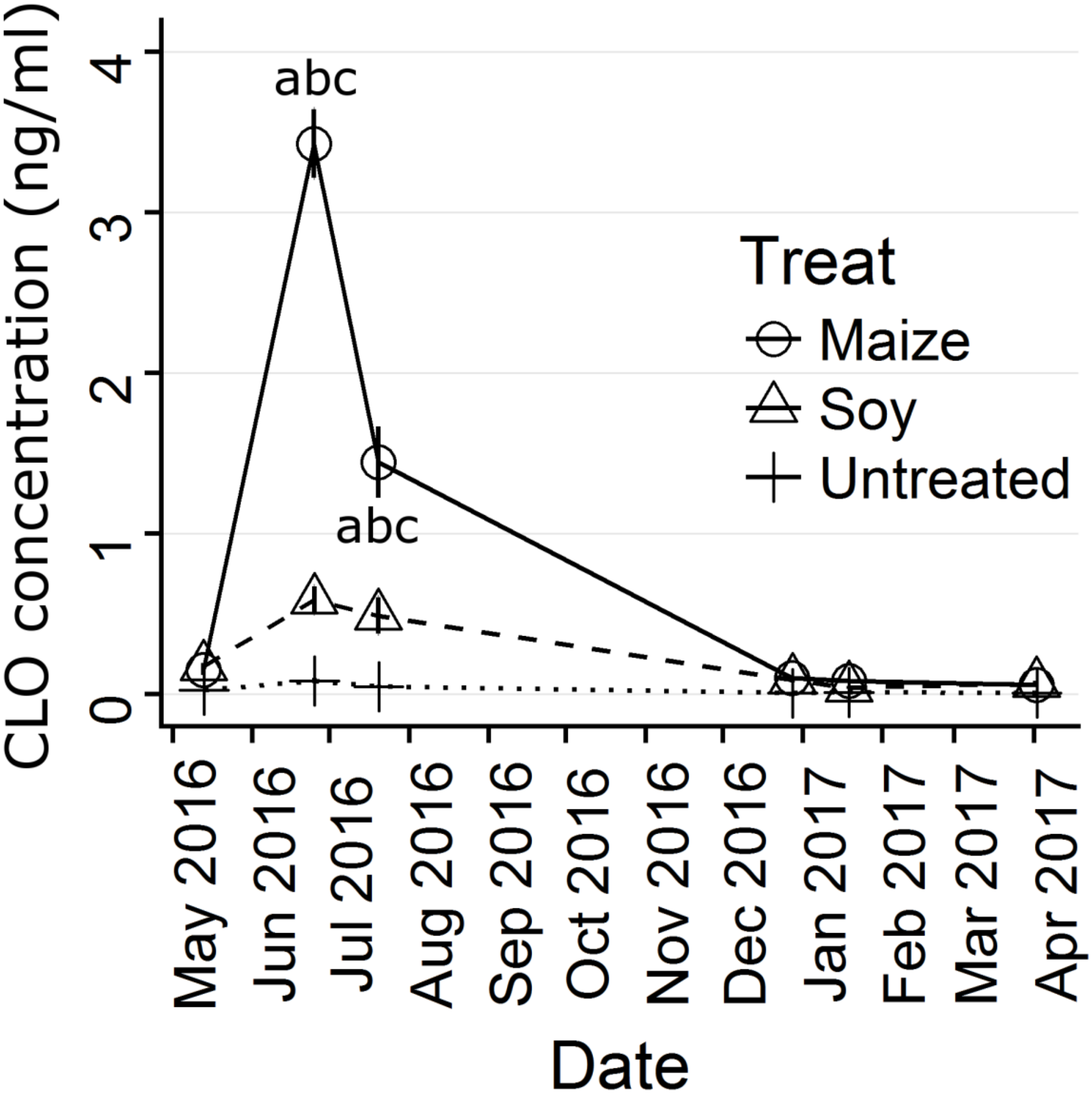


Figure 11. Mean concentration and SE of peak CLO concentrations following selected rainfall events. Sampling events coincided with precipitation events that produced flow in >35 tiles. Groupings were made by initial NST application rate irrespective of tile history. This decision was made after an insignificant time*treatment effect in a multivariate approach to repeated measures model was reported for each grouping. Time points denoted by different letters indicate significant differences ($P = 0.05$) at that sampling event as determined by univariate Tukey comparisons.

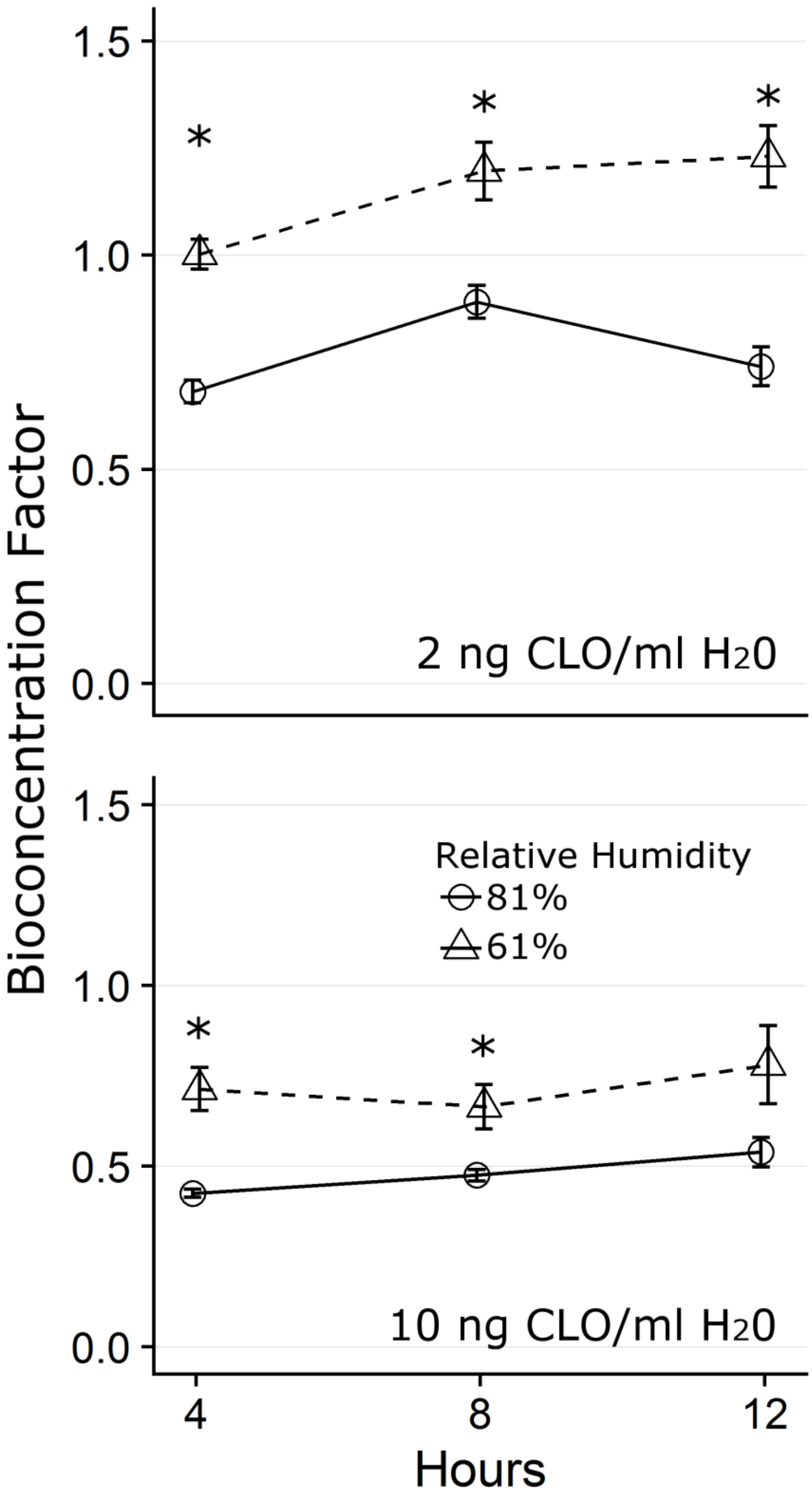


Figure 12. The mean BCF and SE of *L. gibba* grown in half-strength Hutner's media fortified with 2 and 10 ng ml^{-1} CLO solutions at 61% and 81% relative humidity. Time points denoted by * indicate significant differences ($P = 0.05$) at that sampling event.

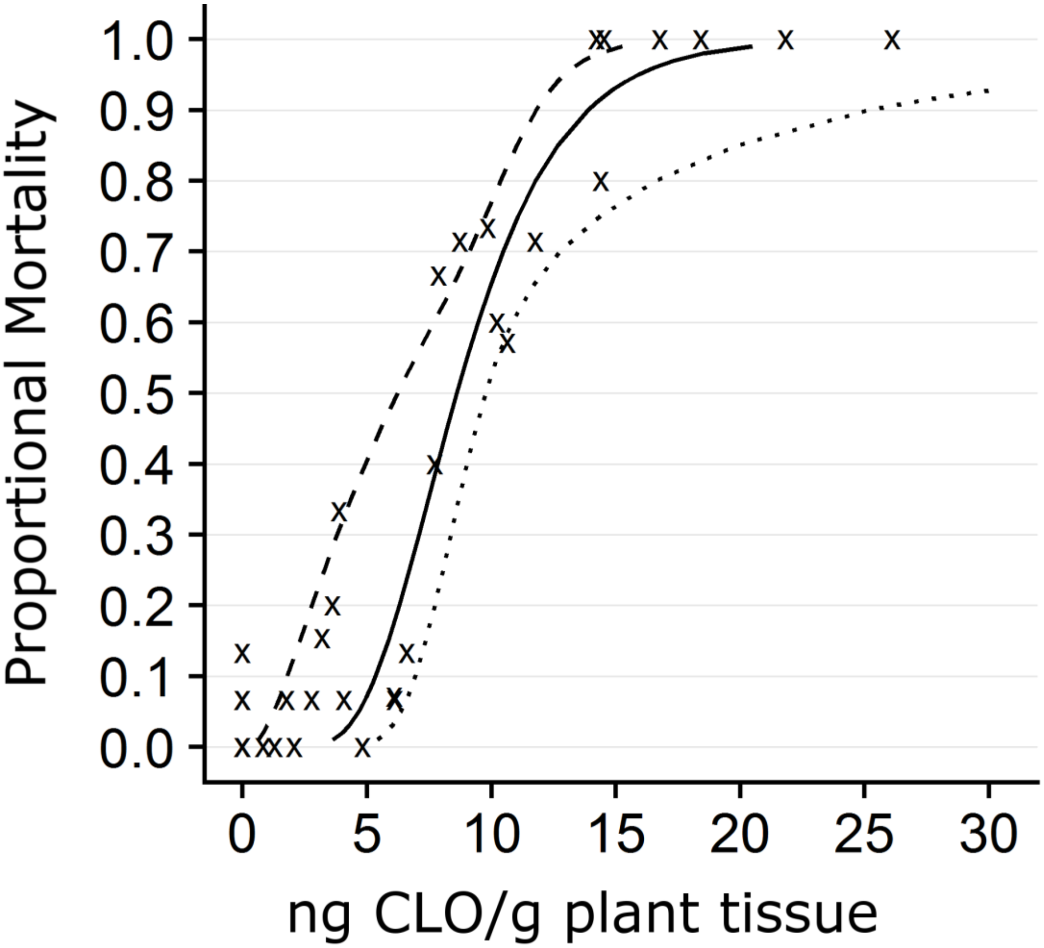


Figure 13. Probit estimated dose response curve for *R. nymphaeae* (solid line) with lower (dashed line) and upper bounds (dotted line) of the 95% fiducial limits. “X” represents uncorrected mortality observations.

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APPENDIX A. MODIFIED QUECHERS PROTOCOL

HOMOGENIZATION

A Precellys® 24-Dual homogenizer along with Precellys® 7 mL homogenization tubes (Bertin Technologies, France) were used to conduct all homogenizations. Up to 1 g of plant material was homogenized in 4 mL of HPLC grade acetonitrile (ACN) solvent. The homogenate was then transferred to a Falcon™ 15 mL conical centrifuge tube and the empty homogenization tube was washed with an additional 4 mL of ACN. The ACN wash was then added to the homogenate followed by an additional 2 mL of ACN to take the total ACN volume up to 10 mL. Cleaning salts (0.5 g Sodium chloride, 2 g magnesium sulfate, 0.25 g sodium hydrogencitrate sesquihydrate) and 10 µL of a 10 ng μL^{-1} d3-clothianidin (Sigma-Aldrich, St. Louis, MO, USA) internal standard were added to the samples which were then centrifuged at 4˚C for 10 min at 2500 rpm. A 1 mL portion of the ACN supernatant was further cleaned in 2 mL QuEChERS dispersive Solid Phase Extraction (dSPE) tubes for use with fruits and vegetables (Agilent Technologies, Santa Clara CA, USA). The dSPE tubes were vortexed for 10 min and centrifuged at 15,000 rpm for 5 min. The ACN supernatant was then transferred to a 1.5 mL Eppendorf tube and concentrated in a SpeedVac until pellet formation, after which the pellet was re-suspended in 100 µL ACN and analyzed with an Agilent 6460 Triple Quadrupole (QQQ) (Santa Clara, CA, USA) using Liquid Chromatography tandem Mass Spectrometry (LC-MS). Clothianidin quantification (Limit of Detection (LOD) 0.1 ng g^{-1}) was achieved by comparing the ratio of deuterium labelled-clothianidin (d3-clothianidin) to that extracted from the plant tissue.

LC/MS/MS SETTINGS

An Agilent 1200 Rapid Resolution liquid chromatography (LC) system coupled to an Agilent 6460 series QQQ mass spectrometer (MS) was used to analyze pesticides in each sample. An Agilent Zorbax SB-Phenyl 4.6 x 150 mm, 5 µm column was used for LC separation (Agilent Technologies, Santa Clara, CA). The buffers were (A) water + 5 mM ammonium acetate + 0.1 % formic acid and (B) acetonitrile (90%) + 5 mM ammonium acetate (10%) + 0.1% formic acid. The linear LC gradient was as follows: time 0 min, 5 % B; time 0.5 min, 5 % B;

time 8 min, 100 % B; time 10 min, 100 % B; time 11 min, 5 % B; time 15 min, 5 % B. The flow rate was 0.8 mL min^{-1}. Multiple reaction monitoring was used for MS analysis. The data were acquired in positive electrospray ionization (ESI) mode. Precursor ions of d3-clothianidin and clothianidin had respective molecular weights of 253 and 249.9 g mol^{-1} with product ions of 172.1 and 169 g mol^{-1}. For both ions, the dwell was set at 50 msec, fragmentor voltage at 70, collision energy at 10V, cell accelerator voltage at 1, with positive polarity. The jet stream ESI interface had a gas temperature of 330°C, gas flow rate of 10 L min^{-1}, nebulizer pressure of 35 psi, sheath gas temperature of 250°C, sheath gas flow rate of 7 L min^{-1}, capillary voltage of 4000 V in positive mode, and nozzle voltage of 1000 V. The ΔEMV voltage was 300. All data were analyzed with Agilent Masshunter Quantitative Analysis (Version B.06.00).

APPENDIX B. META-ANALYSIS SI

Each site/year used in the meta-analyses in Chapter 3 is represented in the following table. Due to the large amount of data used, a variety of abbreviations had to be used. The following will be a list with descriptors for each respective abbreviation and code.

Year (Yr)

Date of planting

Planting location (Loc.): D=DPAC, N=NPAC, P=PPAC, S=SEPAC, T=TPAC

Plot Size: S=Small, L=Large

Hybrid: NK=Northrup King, P=Pioneer, AG=Asgrow, DK=DeKalb, YGRW=Yield Guard Rootworm, Myco=Mycogen

Planting Population (Plant Pop.)

Tillage: CP=Chisel Plow, FC=Field Cultivator, Con=Conventional Tillage, NT=No-Till, D=Disked, RB=Rolling Basket, TT=Tillage Tool, OPT=One-Pass-Tillage Tool, CM=Cultimulcher. If more than one tillage approach was used in a season, the order is represented by entry priority in the table (ex. $1^{st}/2^{nd}/3^{rd}$)

Previous Crop (Prev. Crop): MTC=Maize Trap Crop, M=Maize, SB=Soybean

P250 Std: a “+” designates inclusion in the P250 stand meta-analysis.

P250 3-pt: a “+” designates inclusion in the P250 Oleson scale root damage meta-analysis.

UTC Std: a “+” designates inclusion in the UTC 250 stand meta-analysis

UTC 6-pt: a “+” designates inclusion in the UTC Iowa scale root damage meta-analysis.

UTC 3-pt: a “+” designates inclusion in the UTC Oleson scale root damage meta-analysis.

UTC Yld: a “+” designates inclusion in the UTC yield analysis.

Table 18. Description of datasets used in Chapter 3

Yr	Date of planting	Loc.	Plot Size	Hybrid	Plant Pop.	Tillage	Prev. Crop	P250 Std.	P250 3-pt	UTC Std.	UTC 6-pt	UTC 3-pt.	UTC Yld.
00	5/9/00	D	S	NK58D1	32000	CP/D	MTC			+	+		
00	5/11/00	D	L	P33J24	30000	Con/FC/D	M			+	+		+
00	5/18/00	N	L	Garst 8585 GT	30800	FC	M			+	+		+
00	5/16/00	P	S	NK58D1	32000	CP/D	MTC			+	+		
00	5/17/00	P	L	P3489	29900	CP/D	M			+	+		+
00	5/5/00	S	L	P33Y18	26000	NT	M			+	+		+
00	4/26/00	T	S	NK58D1	32000	CP/D	MTC			+	+		
00	5/11/00	T	L	P34E79	29600	CP/D	M			+	+		+
01	5/9/01	D	S	NK70-D5	27700	CP/D	MTC	+		+	+		
01	5/4/01	D	L	NK70-D5	31000	FC/RB/RB	SB			+	+		+
01	5/10/01	N	S	NK70-D5	27700	CP/FC	MTC	+		+	+		
01	5/10/01	N	L	NK70-D5	33000	CP/FC	M			+	+		+
01	5/3/01	S	L	NK70-D5	28000	NT	M			+	+		+
01	4/30/01	T	S	NK70-D5	27700	CP/D	MTC	+		+	+		
01	5/2/01	T	L	NK70-D5	29600	CP/D	SB			+	+		+
02	5/29/02	D	S	NK N72-V7	27700	CP/D	MTC	+	+	+	+	+	
02	5/29/02	D	L	NK N70-D5	30000	FC	M			+	+	+	+
02	5/30/02	N	S	NK N72-V7	27700	NT	MTC	+	+	+	+	+	
02	5/30/02	N	L	NK N70-D5	33000	NT	M			+	+	+	+
02	5/23/02	P	S	NK N72-V7	27700	Con/CP	MTC	+	+	+	+	+	

02	5/24/02	T	S	NK N72-V7	27700	CP/D	MTC	+	+	+	+	+	
03	4/30/03	N	S	NK N72-V7, DK60-12 YGRW, AG RX708 YGRW	27700	TT/FC	MTC			+	+	+	
03	5/23/03	N	L	NK N70-D5	31800	FC	M			+	+	+	+
03	5/22/03	P	S	NK N72-V7, DK60-12 YGRW, AG RX708 YGRW	27700	Con/CP	MTC			+	+	+	
03	5/22/03	P	L	NK N70-D5	29600	CP/FC	M			+	+	+	+
03	5/2/03	T	S	NK N72-V7, DK60-12 YGRW, AG RX708 YGRW	27700	CP/D	MTC			+	+	+	
03	5/27/03	T	L	NK N70-D5	29600	MBP	SB			+	+	+	+
04	4/29/04	D	S	AG RX708 AG RX708RW	27700	TT/FC	MTC			+	+	+	
04	4/29/04	D	L	AG RX708 AG RX708RW	30000	FC/RB	M			+	+	+	+
04	4/30/04	N	S	AG RX708 AG RX708RW	27700	FC/CM	MTC			+	+	+	
04	4/30/04	N	L	AG RX708 AG RX708RW	30600	FC	M			+	+	+	+
04	5/4/04	P	S	AG RX708 AG RX708RW	27700	Con/CP	MTC			+	+	+	
04	5/5/04	P	L	AG RX708 AG RX708RW	29628	CP	M			+	+	+	+
04	4/28/04	T	S	AG RX708 AG RX708RW	27700	CP, D	MTC			+	+	+	

04	4/28/04	T	L	AG RX708 AG RX708RW	29600	CP/D	SB			+	+	+	+
05	5/24/05	N	S	DK60-16	27700	CP/FC/CM	MTC			+		+	
05	5/25/05	N	L	DK60-16	30800	CP/FC	M			+		+	+
05	5/10/05	P	S	DK60-16	27700	FC/OPT	MTC			+		+	
05	5/16/05	P	L	DK60-16	30813	CP	SB			+		+	+
05	5/4/05	T	S	DK60-16	27700	CP/D	MTC			+		+	
05	5/6/05	T	L	DK60-16	29600	CP/D	SB			+		+	+
06	5/24/06	D	S	DK60-17	27700	D/FC/CP	MTC			+		+	
06	5/24/06	D	L	DK60-17	32000	FC/CP	M			+		+	+
06	5/23/06	N	S	DK60-17	27700	CP/FC/FC	MTC			+		+	
06	5/24/06	N	L	DK60-17	32984	ST	M			+		+	+
06	5/9/06	P	S	DK60-17	27700	OPT/OPT	MTC			+		+	+
06	5/9/06	P	L	DK60-17	30813	OPT/OPT	SB			+		+	+
06	5/6/06	T	S	DK60-17	27700	CP/D/FC	MTC			+		+	+
07	5/8/07	D	S	DK61-73 + fludioxonil + mefenoxam	27700	FC/FC	MTC	+	+				
07	5/9/07	P	S	DK61-73 + fludioxonil + mefenoxam	27700	D/D/OPT	MTC	+	+				
07	5/9/07	P	L	DK61-73 + fludioxonil + mefenoxam	33000	FC	M			+		+	+

07	4/23/07	S	L	DK61-73 + fludioxonil + mefenoxam	29000	NT	M			+		+	+
07	5/7/07	T	S	DK61-73 + fludioxonil + mefenoxam	27700	CP/D/FC	MTC	+	+				
07	5/7/07	T	L	DK61-73 + fludioxonil + mefenoxam	29600	CP/D/FC	SB			+		+	+
08	5/9/08	P	S	DK61-73 + ApronMaxx & P250	27700	OPT	MTC	+	+				
08	5/28/08	T	S	DK61-73 + ApronMaxx & P250	27700	CP/D	MTC	+	+				
09	6/1/09	T	S	Myco 2T780	27700	FC	MTC	+	+				
10	5/5/10	P	S	Myco 2T777	27700	CP	MTC			+		+	
10	4/29/10	T	S	Myco 2T777	27700	CP	MTC			+		+	
11	5/24/11	P	S	SS 7210 RR	27700	FC	MTC	+	+				
11	5/16/11	T	S	SS 7210 RR	27700	FC	MTC	+	+				
12	5/18/12	P	S	P34R65	32000	CP/D/FC	MTC						
12	5/16/12	T	S	P34R65	32000	MBP/FC	MTC						
13	5/14/13	P	S	DK62-95 + Acceleron 500	27700	CP/D	MTC	+	+				
13	5/17/13	T	S	DK62-95 + Acceleron 500	27700	FC	MTC	+	+				

14	5/20/14	P	S	DK61-79 + Acceleron 250	32000	D/FC	MTC	+	+				
14	5/28/14	T	S	DK61-79 + Acceleron 250	27700	D/FC/FC	M	+	+				
15	5/19/15	P	S	Spectrum 6241	30800	OPT/FC	MTC	+	+	+		+	+
15	5/14/15	T	S	Spectrum 6241	30800	FC/FC	M	+	+	+		+	+